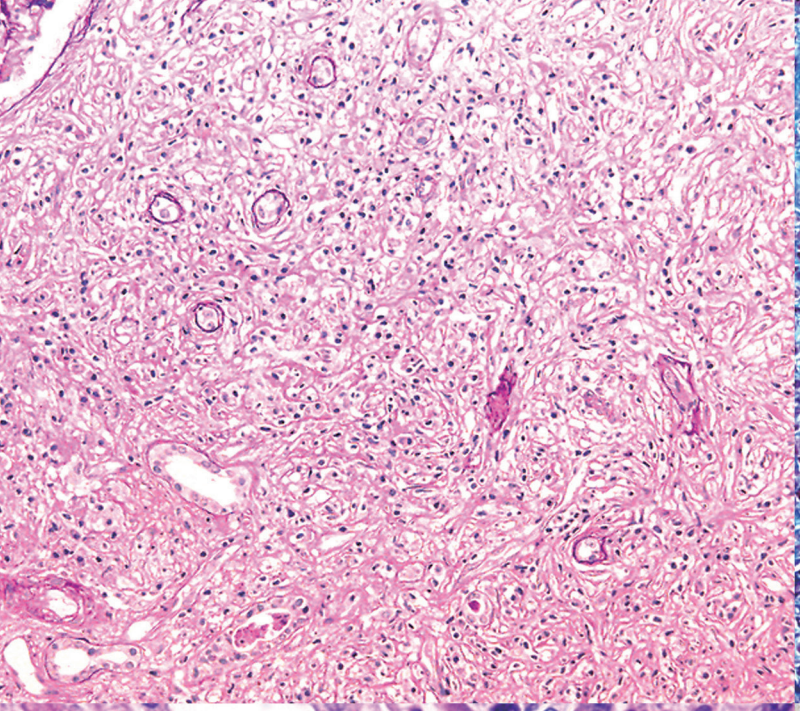
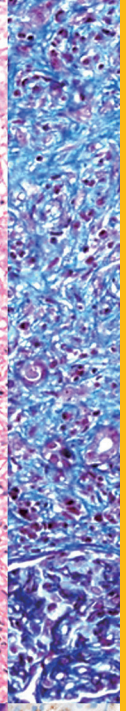
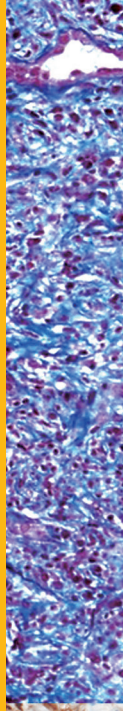
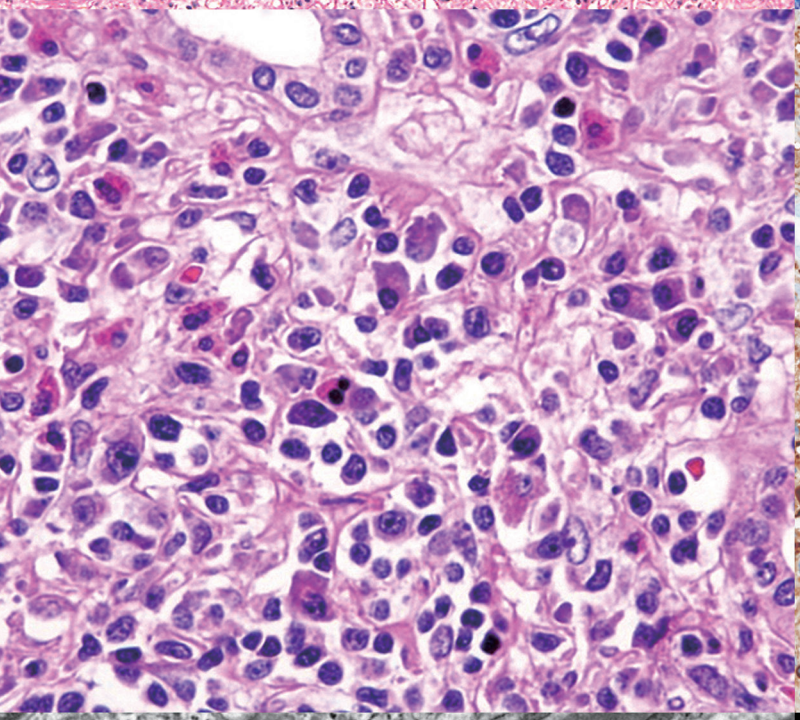
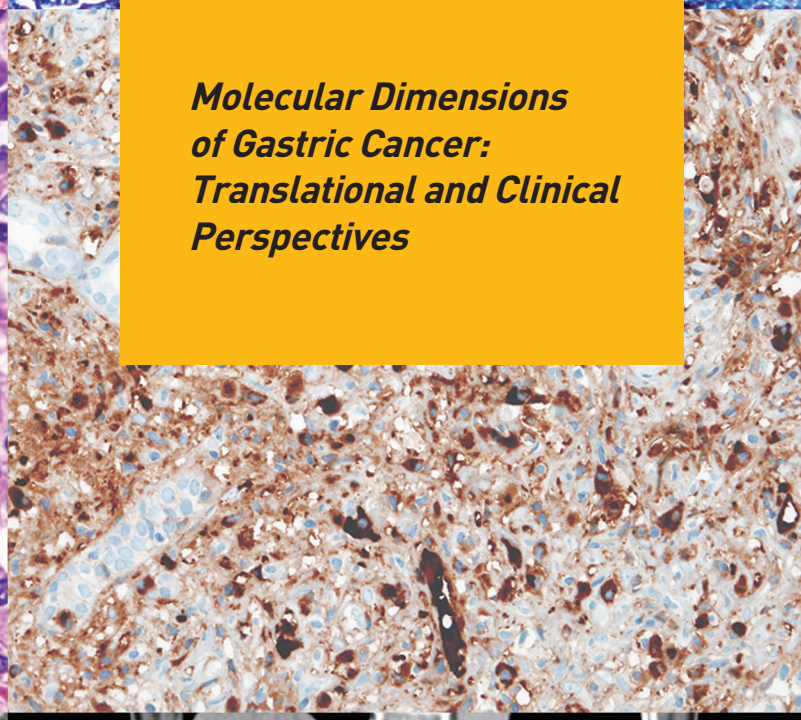
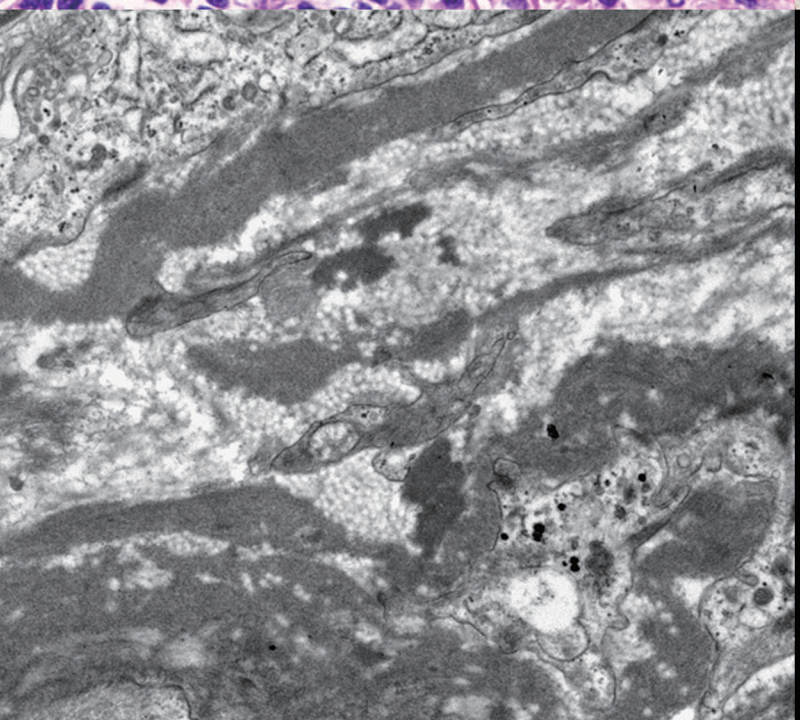
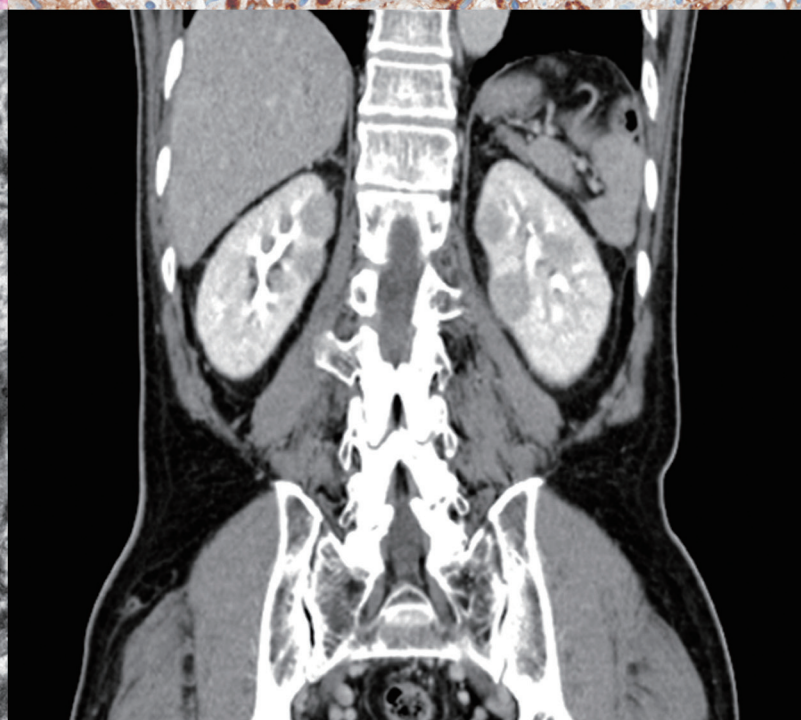

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## *Molecular Dimensions of Gastric Cancer: Translational and Clinical Perspectives*

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REVIEW

# Molecular Dimensions of Gastric Cancer: Translational and Clinical Perspectives

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Gastric cancer is a global health burden and has the highest incidence in East Asia. This disease is complex in nature because it arises from multiple interactions of genetic, local environmental, and host factors, resulting in biological heterogeneity. This genetic intricacy converges on molecular characteristics reflecting the pathophysiology, tumor biology, and clinical outcome. Therefore, understanding the molecular characteristics at a genomic level is pivotal to improving the clinical care of patients with gastric cancer. A recent landmark study, The Cancer Genome Atlas (TCGA) project, showed the molecular landscape of gastric cancer through a comprehensive molecular evaluation of 295 primary gastric cancers. The proposed molecular classification divided gastric cancer into four subtypes: Epstein-Barr virus–positive, microsatellite unstable, genomic stable, and chromosomal instability. This information will be taken into account in future clinical trials and will be translated into clinical therapeutic decisions. To fully realize the clinical benefit, many challenges must be overcome. Rapid growth of high-throughput biology and functional validation of molecular targets will further deepen our knowledge of molecular dimensions of this cancer, allowing for personalized precision medicine.

**Key Words:** Stomach neoplasms; Translational medical research; Cancer genomics; Cancer genetics; Target therapy

Gastric cancer (GC) is one of the most common and fatal diseases, and nearly two-thirds of the cases are concentrated in East Asia.[1] In Korea, GC is the second most common malignancy after thyroid cancer. It was ranked as the third cause of cancer mortality in 2012.[2] An estimate of 35,000 people are expected to be newly diagnosed, and about 7,500 patients will die in 2015.[3] According to the National Cancer Screening Program that began in 1999, the proportion of early gastric cancer (EGC) has increased, and the prognosis of EGC is favorable even without additional treatment after surgery.[4,5] Radical D2 surgery with adjuvant chemotherapy has been established as a standard treatment for locally advanced gastric cancer (AGC), and has improved the prognosis of AGC.[6-8] However, about half of patients with AGC experience recurrence, and the proportion of patients who benefit from adjuvant chemotherapy is around 20%,[9-14] with cases of metastasis being the least amenable to treatment.[15,16]

GC is a heterogeneous malignancy, which is the main reason for its different prognoses in patients with same clinical stage and for its diverse responses to the standard treatment. It is now widely appreciated that genomic complexity and heterogeneity are fundamental causes of tumor phenotypic characteristics determining clinical outcomes. Therefore, understanding of the molecular and genetic characteristics is essential in effective and personalized management of GC.

GC has been classified according to histo-morphologic features. The World Health Organization (WHO) classifies GC into papillary, tubular, mucinous, and poorly cohesive carcinomas,[17] while the Lauren classification divides GC into intestinal, diffuse, and mixed types.[18] However, these classification systems do not satisfactorily provide information relevant to clinical utilities and treatment guidelines. Over the past decades, the molecular landscape of GC has been shaped, and this review focuses on the molecular features of GC that can be translated into clinical use in order to guide precise therapeutic decisions. Details of surgical, adjuvant or peri-operative chemotherapy, and radiotherapy are beyond the scope of this review and readers would be directed to other reviews dealing with these issues.[19] The recent The Cancer Genome Atlas (TCGA) study,[20] a landmark study dividing GC into four subtypes based on multi-dimensional profiling, (1) Epstein-Barr virus (EBV) tumor, (2) microsatellite unstable

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(MSI) tumor, (3) genomically stable (GS) tumor, and (4) chromosomal instability (CIN), is used as a roadmap for this review. We also provide the current status of recent and ongoing clinical trials that focus on genomic alterations in molecular subtypes for targeted therapeutics (summarized in Table 1).[15,16,21-34]

## EPSTEIN-BARR VIRUS–POSITIVE GASTRIC CANCER

The incidence of EBV-positive GC has been reported to be around 10%[20,35] and harbors a higher prevalence of DNA hypermethylation than other subtypes.[20,36] The reason for extraordinary DNA hypermethylation seems to be a cellular reaction to the viral infection.[37] EBV-positive GC also has strong signatures of interleukin 12-mediated signaling events, which reflects high immune cell infiltration.[20,38] Intriguingly, tumors of this subtype exhibit *CD274* and *PDCD1LG2* amplification of which proteins PD-L1 and PD-L2, respectively, are related to immune suppressive functions, particularly immune checkpoints. Further, amplification at the 9p24.1 locus containing *JAK2*, which encodes an oncogenic receptor tyrosine kinase (RTK), is also a potential therapeutic target. Those findings seemed to support the rationale that this type of GC would be a relevant target of RTK and immune checkpoints inhibitors.[20,37] A recent phase I study showed that pembrolizumab (MK-3475), one of the anti–PD-1/2 immune checkpoint inhibitors, provided antitumor activity in patients with AGC that expressed PD-L1.[21] Predilection of EBV-positive GC for *PIK3CA* mutation, which is related to the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, has also been reported.[20] Frequent *PIK3CA* mutations warrant evaluation of PI3K inhibitors in EBV-positive GC. Although still in preclinical stages, BKM 120, which is a direct PIK3CA inhibitor, and BEZ235, a dual PIK3CA and mammalian target of rapamycin (mTOR) inhibitor, have been reported to reduce cell viability and induce apoptosis in GC cell lines.[22] Additionally, the *ARID1A* mutation was detected in 10% of GC[39] and was

**Table 1.** Current status of targeted therapies based on molecular alterations according to GC subtype

| Subtypes | Molecular targets | Alterations | Suggested therapeutics | Clinical trial |
|---|---|---|---|---|
| EBV positive GC | PD-L1/2 | Overexpression | Pembrolizumab | Phase I[21] |
| | | | | |
| | PIK3CA | Mutation | BKM120/BEZ235 | Preclinical/Preclinical[22] |
| | ARID1A | Mutation | NA | NA |
| MSI GC | MMR | Deficiency | Pembrolizumab | Phase II[23] |
| GS GC | CDH1 | Mutation | Prophylactic gastrectomy (germline mutation)[24,a] | - |
| | RHOA | Mutation | NA | NA |
| | CLDN18-ARHGAP | Fusion | NA | NA |
| CIN GC | TP53 | Mutation | NA | NA |
| | SMAD4 | Mutation | NA | NA |
| | APC | Mutation | NA | NA |
| | EGFR | Overexpression | Cetuximab | Phase III (EXPAND trial, negative)[25] |
| | EGFR | Overexpression | Panitumumab | Phase III (REAL-3 trial, negative)[26] |
| | HER2 | Overexpression | Trastuzumab | Phase III (ToGa trial, approved)[16] |
| | HER2 | Overexpression | Trastuzumab | Phase III (HELOISE trial, ongoing NCT01450696) |
| | HER2 | Overexpression | Pertuzumab | Phase III (JACOB trial, ongoing NCT01774786) |
| | HER2 | Overexpression | Trastuzumab emtansine | Phase II/III (GATSBY trial, ongoing NCT01641939) |
| | EGFR/HER2 | Overexpression | Lapatinib | Phase III (TyTAN trial, negative)[27] |
| | MET | Overexpression | Crizotinib/rilotumumab | Phase I/Phase II (terminated)[28-31,b] |
| | MET | Overexpression | Onartuzumab | Phase III (METGASTRIC trial, ongoing NCT01662869) |
| | VEGF | Overexpression | Bevacizumab | Phase III (AVAGAST trial, negative)[15,32] |
| | VEGFR2 | Overexpression | Ramucirumab | Phase III (REGARD[33] & RAINBOW[34] trials, approved) |
| | VEGFR2/TIE2 | Overexpression | Regorafenib | Phase II (INTEGRATE trial, ongoing ACTRN12612000239864) |

GC, gastric cancer; EBV, Epstein-Barr virus; N/A, not available; MSI, microsatellites unstable; GS, genomically stable; CIN, chromosomal instability.
[a]Mainly in the Western countries; [b]Amgen-sponsored clinical trials of rilotumumab in advanced gastric cancer were terminated based on the pre-planned safety review by independent data monitoring committee.


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found most frequently in EBV-positive GC.[20] This mutation encodes a component of SWI/SNF complex and acts as a tumor suppressor in cancer.[39] Recently, it was reported that EZH2 inhibitor could be a novel therapeutic targeting *ARID1A*-mutated cancers.[40] Therefore, the *ARID1A* mutation provides another clinically actionable genetic alteration in EBV-positive GC that should be validated in a clinical study. Based on these findings, the molecular characteristics of EBV-positive GC are distinct from those of other GC subtypes, and some of the genetic alterations can be therapeutically exploited.

## MICROSATELLITE UNSTABLE GASTRIC CANCER

MSI GC is related to the loss of function of mismatch repair (MMR) genes and is associated with older age, female gender, intestinal type, and less aggressive tumor stages.[20,41,42] Because the function of the MMR mechanism is defective mainly due to *MLH1* silencing by promoter hypermethylation,[20] this subtype has more mutations per megabase (Mb) compared to other types of GC. Intriguingly, MSI tumors possess common alterations in major histocompatibility complex (MHC) class I-related genes, including *HLA-B* and *B2M*. Since these MHC class I genes function in proper antigen presentation to the host immune system, these genomic alterations could provide hypermutated MSI GC with the selective advantage of immune surveillance evasion.

The incidence of MSI GC was previously reported to be 8.5%–37.8%.[43] While the prognosis of MSI GC was not assessed in comparison with those of other molecular subtypes in a TCGA study,[20] a meta-analysis[43] and a recent study regarding the molecular classification of GC reported that MSI GC had the best overall prognosis with the lowest recurrence rate.[44] Importantly, the prognosis of MSI GC was prominent in the population treated with surgery alone. Indeed, the prognosis of MSI GC without chemotherapy was similar to that of patients who received chemotherapy after surgery,[45] implying that the MSI subtype is unresponsive to chemotherapy in an adjuvant setting,[41] like MSI colon cancer.[46,47] Additionally, a recent phase II study that evaluated the clinical utility of pembrolizumab showed that MMR status predicted the benefit of pembrolizumab,[23] and a higher mutational load was reported to be related to positive response to anti-CTLA-4 in melanoma[48] and PD-1 antibody in non-small cell lung cancer.[49] The legitimate explanation might be that immune infiltrate related with mutation was directed at neoantigens, and recognition plays an important role in the antitumor immune response.[23] Consequently, MSI status is a promising biomarker to predict the prognosis and responses to immune checkpoint inhibitor as well as chemotherapy in GC, as in colon cancer.[50] There is no consensus on the definition of GC-specific MSI in clinical settings at this time, and studies have used different criteria to define MSI.[20,43,45,51,52] Thus, it is necessary to establish appropriate analysis standards for MSI status in GC for precise detection and translation of "MSI-ness" for clinical therapeutic decisions.

## GENOMICALLY STABLE GASTRIC CANCER

The GS subtype of GC is best represented as a diffuse type of GC, with lower mutation burden compared to other subtypes and occurring at a relatively early age.[20] *CDH1* mutation is one of the representative mutations in the GS subtype. *CDH1* germline mutations are known to be related to hereditary diffuse GC. When patients harbor pathogenic hotspot mutations in *CDH1*, prophylactic gastrectomy is recommended.[24] However, only two *CDH1* mutations, neither of which is a pathogenic hotspot mutation, were identified in a recent TCGA study.[20] Another study reported that somatic alterations of *CDH1* were present in approximately 30% of GC cases, and structural alterations in *CDH1* were related to poor prognosis.[53] In addition to *CDH1* mutations, GS subtype tumors have *RHOA* mutations and *CLDN18-ARHGAP 6* or *26* fusions.[20,54,55] *RHOA* is known to modulate downstream Rho signaling, and its mutation imparts resistance to anoikis, a form of programmed cell death.[54] Also, *RHOA* acts to control actin-myosin-dependent cell contractility and motility;[56,57] thus, its mutation might contribute to dispersed growth and poorly cohesive patterns of diffuse type GC,[20] which is associated with poor prognosis. Thus, the *RHOA* mutation could be a good candidate for new approaches targeting GS subtype GC.[37] *CLDN18-ARHGAP6* or *26* fusions are mutually exclusive to *RHOA* and *CDH1* mutation among GS tumors. The discovery of recurrent interchromosomal translocation between *CLDN18* and *ARHGAP26* further implies biological significance of cell adhesion and deregulated Rho signaling in GS tumors since CLDN18 is involved in intercellular tight junction structure, and ARHGAP26, a GTPase-activating protein, imparts Rho signaling activation by facilitating the conversion of Rho GTPases to the GDP state. A recent study reported that this type of fusion in epithelial cells mediates epithelial disintegration and is related to epithelial-mesenchymal transition (EMT).[58] Therefore, the novel discoveries of *RHOA* mutation and *CLDN18-ARHGAP26* fusion could be exploited to develop new therapeutic strategies against GS subtype tumors,[37] which are known

to harbor the poorest prognosis of all GC tumors.[44] However, translating those new strategies to clinical practice is in the early stages and is largely lacking evidence of functional validity. Additionally, there have been no clinical trials to assess the efficacy of targeting those genomic alterations in GS subtype tumors.

## CHROMOSOMAL INSTABILITY GASTRIC CANCER

CIN subtype GC is related to intestinal type histology, frequent *TP53* mutations, and amplification of RTKs.[20] *TP53* mutation is the most frequently detected mutation in GC, occurring in up to 50% of all cases[59] and 71% of cases of CIN subtype GC.[20] *TP53* mutation is associated with high levels of somatic copy number variations in both chromosomal and focal gene regions.[60] Also, other canonical tumor suppressor genes such as *SMAD4* and *APC* have been reported to be mutated in GC.[61] Since tumor suppressor genes are regarded as poor candidates for targeted therapy development, alterations in RTKs will be discussed in this review.

## EPIDERMAL GROWTH FACTOR RECEPTOR

The human epidermal growth factor receptors (HER) are a family of four transmembrane RTKs, ErbB1 (epidermal growth factor receptor, EGFR), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4),[62] that regulate diverse downstream signaling pathways and play an important role in GC development and progression. A biomarker study from Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC),[14] a phase III randomized controlled trial (RCT) that compared the effect of adjuvant S-1 over surgery alone in locally AGC, showed that EGFR overexpression was related to poor prognosis but was not found to be the case for HER2.[63] There have been two RCTs that investigated the benefit of EGFR inhibition (EXPAND[25] trial for cetuximab and REAL-3 trial[26] for panitumumab as first-line therapy); however, both trials failed to prove the additional clinical benefit of anti-EGFR antibody over standard chemotherapy.

## HER2

The success of a clinical trial that investigated the effects of trastuzumab targeting HER2 in HER2-overexpressed GC patients resulted in changes in clinical practice. Addition of trastuzumab to chemotherapy as the first-line treatment of metastatic GC improved overall survival.[16] However, an updated survival analysis showed that the benefit of the trastuzumab decreased over time, the difference in median overall survival was reduced from 2.7 to 1.4 months, and the hazard ratio increased from 0.74 in primary analysis to 0.80.[64] This raises a concern, requiring further investigation to clarify the clinical benefit of trastuzumab in HER2-overexpressing GC patients. Indeed, two first-line therapy trials are underway to investigate the effect of addition of pertuzumab to a standard HER2 targeting regimen and the effect of two dose levels of trastuzumab (JACOB and HELIOSE, respectively).

The frequency of *HER2* mutation was reported to be 5% (9/180) in GC, and the relationship between *HER2* mutation and responsiveness to trastuzumab has not yet been determined. Another clinically important issue regarding *HER2* mutation and amplification might be derived from a recent EGFR biomarker study in non-small cell lung cancer patients receiving gefitinib, where *EGFR* mutation and amplification correlated with prolonged progression-free survival.[65] Based on this, it might be worthwhile to investigate the clinical benefit of HER2 inhibitor in *HER2*-mutated and amplified GC as alternative candidates for HER2-targeted therapy.[66] A phase III trial (TYTAN) was conducted to investigate the benefit of lapatinib, a dual inhibitor of EGFR and HER2, as a second-line therapy for AGC. Although overall survival was not significantly different, *post hoc* analysis demonstrated that the HER2 immunohistochemistry 3+ subgroup showed statistically significant prolongation of overall survvial.[27]

## KRAS

*KRAS* is one of the members of the RAS family, and its mutation plays an important role in tumorigenesis by activating downstream pathways such as PI3K and RAF. The frequency of *KRAS* mutations in GC was reported as 1.5%–5.8%, and most of them were transversions.[67] Overexpression of wild-type *KRAS* seemed to be related to acquired resistance to inhibitors of other tyrosine kinase in GC cells.[68] A phase II trial that evaluated the efficacy of selumetinib, an inhibitor of MEK1/MEK2, downstream of KRAS, for *KRAS*-mutant non-small cell lung cancer demonstrated promising efficacy and thereby warrants further clinical investigation.[69] Thus, a MEK inhibitor could be a potential therapeutic agent for targeting *KRAS*-mutated GC; however, evidence from a clinical trial is required.


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## MESENCHYMAL EPITHELIAL TRANSITION FACTOR

Mesenchymal epithelial transition factor (*MET*) amplification was not common (2%, 10/489) in GC it was reported to be related with poor prognosis.[28] However, two studies have reported the possibility of targeted therapy for MET-positive GC. An expanded phase I cohort study showed that patients with *MET* amplification had a favorable response to crizotinib (PF-02341066), a MET/anaplastic lymphoma kinase tyrosine kinase inhibitor.[28] Furthermore, rilotumumab (AMG 102), a fully humanized monoclonal antibody against hepatocyte growth factor/MET, demonstrated favorable overall survival especially for patients with MET-positive GC.[29] Based on those results, subsequent trials were conducted (RILOMET-1[30] and NCT02137343); however, all Amgen-sponsored clinical trials of rilotumumab in AGC were terminated based on a pre-planned safety review by the data monitoring committee due to an increase in death with the study drug.[31] Currently, a small-molecule MET inhibitor is under investigation for MET-amplified GC.

### *BRAF*

*BRAF* mutations are related to tumorigenesis, and dysregulated BRAF activity instigates abnormal cell growth and proliferation through MEK and ERK pathways.[70] The specific mutation $BRAF^{V600E}$ is the most common type of *BRAF* mutation in melanoma, and vemurafenib, a BRAF inhibitor, was found to be beneficial in patients with $BRAF^{V600E}$-mutated melanoma.[71] *BRAF* mutations are rarely observed in GC, with only 2.2% (7/319) of patients demonstrating *BRAF* mutation, most (five of seven *BRAF* mutations) of which were $BRAF^{V599M}$;[72] in addition, there were no *BRAF* mutations among 167 patients in the REAL-3 trial.[26] Furthermore, only 0.2% of patients (1/508) with GC had a $BRAF^{V600E}$ mutation in another study.[67] Therefore, it is not yet clear if *BRAF* mutation is a driver mutation in GC.

## VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR

Vascular endothelial growth factor receptor (VEGFR) expression is closely related to angiogenesis in tumorigenesis. Since angiogenesis is critical for tumor growth and metastasis, it is a therapeutic target for many cancer types. Bevacizumab, a monoclonal antibody that inhibits VEGF (VEGF-A), showed survival benefits in advanced colorectal cancer[73] and non-small cell lung cancer.[74] Also, a phase II trial that showed efficacy of bevacizumab for advanced gastro-esophageal cancer seemed to reinforce the success of targeted therapy against the VEGF pathway in gastric cancer.[75] Despite initial enthusiasm regarding its use, bevacizumab combined with chemotherapy as a first-line therapy did not improve the overall survival of patients with GC in the AVAGAST trial. However, the more recent RAINBOW[34] and REGARD[33] trials, which evaluated benefits of ramucirumab (antibody targeting VEGFR2) as second-line and first-line therapies, respectively, reported improved overall survival in GC patients. Intriguingly, subgroup analyses of the trials showed that benefit from VEGF-targeted therapy was observed mainly in non-Asian patients. Also, a subsequent biomarker study of AVAGAST showed that plasma VEGF-A and neuropilin-1 levels could be prognostic and predictive of bevacizumab treatment in a non-Asian population.[32] Those findings imply that GC is a complex and heterogeneous disease across the globe, which affects the response to anti-angiogenesis treatment and potentially other target therapies.[37,76]

## CANCER STEM CELL-RELATED PATHWAYS

Cancer stem cells that initiate tumorigenesis through self-renewal and differentiation are emerging concepts in cancer research. Such cells activate EMT, oncogenic pathways, and embryogenic pathways.[77,78] Also, these cells are known to be resistant to chemotherapy and radiotherapy, while Wnt, Notch, and Hedgehog pathways are crucial to the maintenance of cancer stem cells. Transcriptional factors such as Snail, Slug, Twist, and Zeb1/2 coordinate the EMT, while transforming growth factor β (TGF-β) is a central signaling pathway related to transformation into EMT.[79,80] TGF-β can act as a proto-oncogene, driving matrix deposit, stimulating EMT and stem cell renewal, and inhibiting apoptosis through transactivation of EGFR.[81,82] Also, its downstream signaling pathway, PI3K/Akt/mTOR, aids in cancer stem cell maintenance.[83] Thus, targeting this pathway with appropriate agents such as metformin would inhibit cellular transformation and selectively kill cancer stem cells, as previously demonstrated in breast cancer.[84] Also, a recent report showed that patients with GC treated with metformin for diabetes mellitus had better survival compared to those treated with diabetic medications other than metformin.[85] GC stem cells express CD133, CD44, aldehyde dehydrogenase 1 (ALDH1), and ATP-binding cassette sub-family G member 2 (ABCG2). CD44 and ALDH1 have been reported to be related to resistance to chemotherapy and radiotherapy.[86] Amplification of the gene that en-

codes the GC stem cell marker CD44 was observed in a TCGA study,[20] suggesting the potential of exploiting genomic alteration in future development of cancer stem cell-directed therapies.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Through extraordinary efforts over the past decades, our knowledge on GC has advanced considerably, and standardized multidisciplinary treatment has improved the prognosis of GC. However, clinical development of targeted therapy in GC remains inferior to those of other cancer types such as lung, breast, and colon cancer in terms of genetic sequencing and molecular therapeutics.[87] Most of the targeted therapies have been investigated without patient selection based on a biomarker, and the results have been disappointing, with only a few targeted agents[16,33,34] showing benefit to patient survival at one year even after treatment for metastasis or recurrent GC. In the upcoming years, accumulation of genomic information and knowledge about molecular pathogenesis of GC will be accelerated through high-throughput systems biology, and the treatment will be focused on targeting specific GC subtypes based on specific molecular characteristics (e.g., somatic driver alterations and amplification). Presently, one of the hurdles in this achievement is the integration of knowledge from various disciplines and its translation into daily clinical practice. To achieve a sensible reduction in mortality due to this deadly disease, transdisciplinary cooperation among clinicians, pathologists, bioiformaticians, computational biologists, and genomicists is required.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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REVIEW

# Ménétrier's Disease: Its Mimickers and Pathogenesis

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Ménétrier's disease is a rare protein-losing hypertrophic gastropathy. Histologically, it can be mistaken for other disorders showing hypertrophic gastropathy. The pathogenesis of Ménétrier's disease is not fully understood; however, it appears that the epidermal growth factor receptor (EGFR) ligand, transforming growth factor alpha, contributes to the pathogenesis of this disorder. In this review, we will discuss disease entities that can mimic Ménétrier's disease and the role of EGFR signaling in Ménétrier's disease.

**Key Words:** Ménétrier's disease; Gastric polyp; Transforming growth factor alpha

Ménétrier's disease (MD), hypoproteinemic hypertrophic gastropathy, is a rare acquired disorder characterized by giant gastric rugal folds in the body and fundus, often with antral sparing, decreased acid secretion, increased gastric mucus production, and hypoalbuminemia secondary to protein loss in the gastric mucosa.[1] It affects men more frequently than women, and the typical age at diagnosis is between 30 to 60 years.

## CLINICAL PRESENTATION

Typical clinical presentations include nausea, vomiting, diarrhea, abdominal pain, weight loss, malnutrition, and peripheral edema due to hypoalbuminemia. MD usually shows an insidious onset with progressive features and is associated with an increased risk of gastric cancer.[2,3] Variants with abrupt onset and spontaneous remission have been reported. These variants are associated with cytomegalovirus (CMV) or *Helicobacter pylori* infection.[4-6] CMV-associated cases usually occur in children; however, cases in adults have also been reported.[7-10] MD has also been reported in patients with autoimmune diseases such as inflammatory bowel disease, sclerosing cholangitis, and ankylosing spondylitis, suggesting an immunological component to its pathogenesis.[11,12]

Endoscopically, the markedly thickened gastric mucosal folds resemble cerebral convolutions and primarily affect the body and fundus but spare the antrum (Fig. 1). Gastric pH is increased due to loss of parietal cells, and copious thick mucus production is seen secondary to foveolar hyperplasia.[2,3]

## MICROSCOPIC FEATURES

Foveolar hyperplasia that often results in mucosal thickness 1 cm or greater is the most striking feature of MD. The foveolar epithelium shows a corkscrew morphology due to massive foveolar hyperplasia, but the overall linear architecture is maintained (Fig. 2A). Oxyntic glands are atrophic with reduced or absent parietal cells, and deep glands can be cystically dilated. The lamina propria shows a variable amount of predominantly chronic inflammatory cell infiltration with scattered eosinophils. Prominent vertical strands of smooth muscle in the lamina propria are also identifiable (Fig. 2D).

## DIFFERENTIAL DIAGNOSIS

Diseases that show thickened gastric folds can mimic MD at





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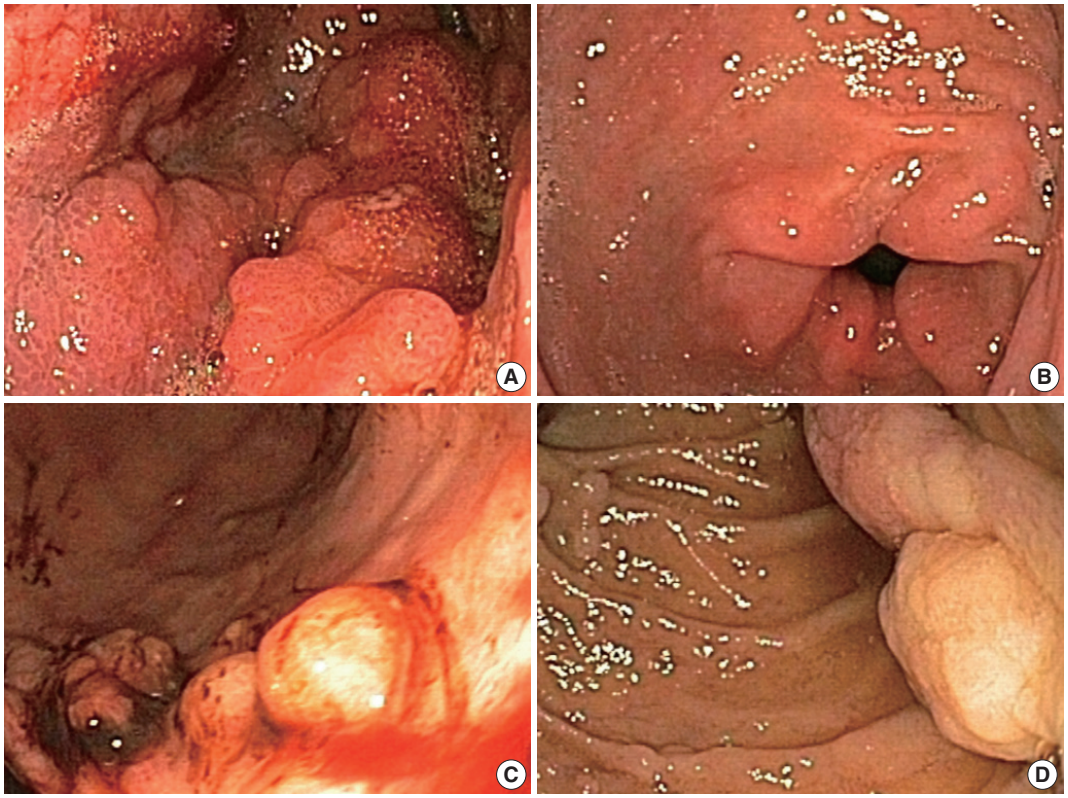


**Fig. 1.** Endoscopic appearance of Ménétrier's disease and juvenile polyposis syndrome. (A) Gastric body of Ménétrier's disease patient with diffuse hypertrophic gastric folds. (B) Gastric antrum of the same patient is not involved. (C) Gastric body of juvenile polyposis syndrome patient with multiple sessile polyps. (D) Juvenile polyps in the duodenum.

endoscopy. These conditions range from foveolar hyperplasia in reactive conditions to malignancy (Table 1). Because some of these diseases share histologic characteristics with MD, correlation of microscopic findings with endoscopic and clinical features is important in order to establish the correct diagnosis.

Because accurate diagnosis of some of these diseases requires examination of very thick gastric mucosa, large snare biopsies that capture the entire thickness of the mucosa are recommended instead of standard forceps biopsies. Disease entities with diffuse hypertrophic gastropathy include hypertrophic lymphocytic gastritis, hypertrophic hypersecretory gastropathy, and Zollinger-Ellison syndrome (ZES). Hypertrophic lymphocytic gastritis often presents as giant fundic mucosal folds with relative sparing of the antrum similar to MD. In contrast to MD, the gastric mucosa in this condition shows diffuse and severe inflammation with prominent intraepithelial lymphocytes. Foveolar hyperplasia is confined to areas with inflammation.[13] Hypertrophic hypersecretory gastropathy is a rare acquired gastropathy that involves hypersecretion of acid, pepsin, and mucin. Endoscopically, it is characterized by hypertrophic gastric folds and "cobblestone" gastric body mucosa with atrophic antral mucosa. Histologically, it is differentiated from MD in that hyperplasia is seen in both the foveolar epithelium and oxyntic glands. Gastric glands with cystic dilatation can also be seen.[14,15] ZES is characterized by ectopic gastrin secretion (gastrinoma), increased gastric acid secretion, and intractable peptic ulcer disease, and it shows diffusely thickened gastric folds, especially in the body and fundus. Multiple peptic ulcers can also be observed. Histologic characteristics of ZES include diffuse parietal cell hyperplasia and hypertrophy. Parietal cells extend to the base of the glands and into the antrum. However, the foveolar epithelium does not show hyperplasia. Nodular and linear enterochromaffin-like cell hyperplasia can frequently be observed in the disease and is associated with multiple endocrine neoplasia type 1.

Gastric polyps can manifest as focal hypertrophic gastropathy and can be numerous and diffusely distributed in polyposis syndromes, resulting in a hypertrophic appearance. Gastric hyperplastic polyps are the most common, accounting for 70% of gastric epithelial polyps.[16] The antrum is the most common site for hyperplastic polyps; however, they can develop in other areas of the stomach and can be solitary or multiple. Hyperplastic polyps usually develop in the background of other gastric patholo-

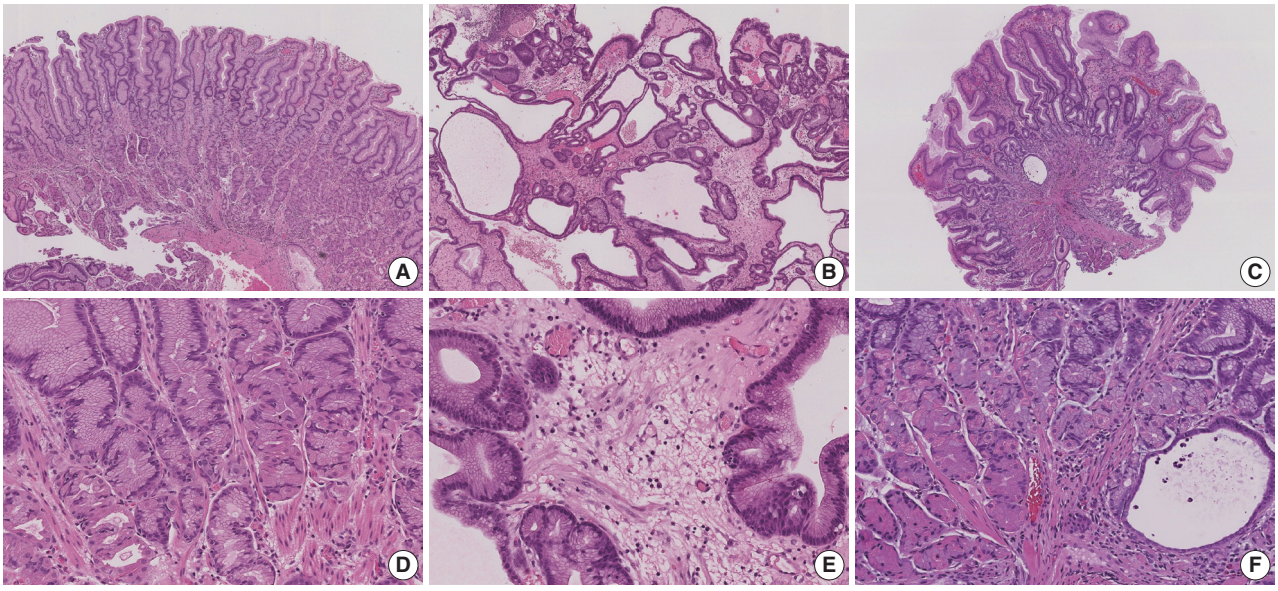


**Fig. 2.** Histologic comparison of Ménétrier's disease and gastric polyps. (A) Ménétrier's disease shows foveolar hyperplasia with corkscrew morphology and cystically dilated deep glands; however, overall linear architecture is maintained. (B) Juvenile polyp shows foveolar hyperplasia with cystically dilated superficial and deep glands. Linear architecture is disrupted. (C) Peutz-Jeghers polyp shows foveolar hyperplasia and cystically dilated glands. Lamina propria of Ménétrier's disease shows strands of smooth muscle bundles (D) whereas lamina propria of juvenile polyp is edematous without prominent smooth muscle (E). (F) Peutz-Jeghers polyp shows arborizing smooth muscle strands in the lamina propria, which is less prominent than counterparts in small intestine or colon.

**Table 1.** Differential diagnosis of Ménétrier's disease

| Diagnosis | Distribution | Location in stomach | Hyperplastic mucosal compartment | Pathologic features |
|---|---|---|---|---|
| Ménétrier's disease | Diffuse | Body and fundus; relative sparing of antrum | Foveolar epithelium | Massive foveolar hyperplasia |
| Hypertrophic lymphocytic gastritis | Diffuse | Body and fundus; relative sparing of antrum | Foveolar epithelium | Prominent intraepithelial lymphocytes |
| Hypertrophic hypersecretory gastropathy | Diffuse | Body and fundus; atrophic antrum | All layers | Hyperplasia of all glandular compartments |
| Zollinger-Ellison syndrome | Diffuse | Body and fundus | Parietal cells | Parietal cell hyperplasia |
| Hyperplastic polyp | Focal | Antrum; body and fundus also possible | Foveolar epithelium | Foveolar hyperplasia with architectural distortion |
| Polyposis syndrome with hamartomatous polyps | Variable | Body, fundus, and antrum | Foveolar epithelium | Features similar to hyperplastic polyp |
| Gastric adenocarcinoma and proximal polyposis of the stomach | Variable | Body and fundus | Oxyntic glands | Fundic gland polyps with low and high-grade dysplasia |
| Diffuse gastric carcinoma | Variable | Body, fundus, and antrum | Not applicable | Infiltrating carcinoma; diffuse type |
| Lymphoma | Variable | Body, fundus, and antrum | Not applicable | Effacement of gastric mucosa by infiltrating lymphoma cells |
| Amyloidosis | Variable | Body, fundus, and antrum | Not applicable | Acellular, amorphous eosinophilic material surrounding glands and vessels |

gy such as atrophic gastritis, reactive gastropathy, or any form of acute or chronic gastritis. The polyps show foveolar hyperplasia with dilated and tortuous glands. Foci of intestinal metaplasia can be seen in 15% of lesions. Surface erosions and ulcerations are frequently observed and associated with reactive changes.

Juvenile polyposis syndrome (JPS) is characterized by hamartomatous polyps in the gastrointestinal tract, most commonly in the colon. Most colonic juvenile polyps are sporadic, but germline mutations in either *BMPR1A* or *SMAD4* genes have been detected in 50% of cases of JPS, which is inherited in an autosomal dominant pattern. A severe gastric phenotype has been shown in *SMAD4* mutations, particularly nonsense mutations


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**Table 2.** Microscopic features of Ménétrier's disease, hyperplastic polyp, and juvenile polyp in the gastric body/fundus

| Feature | Ménétrier's disease | Hyperplastic polyp | Juvenile polyp |
|---|---|---|---|
| Foveolar epithelium | Massive foveolar hyperplasia and surface erosion | Foveolar hyperplasia and surface erosion | Foveolar hyperplasia, ulceration, reactive changes |
| Oxyntic glands | Decreased number of parietal and chief cells | Unremarkable | Unremarkable |
| Glandular architecture | Tortuous foveolar epithelium and cystically dilated deeper glands; overall linear architecture is maintained | Cystically dilated and disorganized foveolar epithelium; linear architecture is maintained in deeper glands | Cystically dilated and disorganized foveolar epithelium and deeper glands |
| Lamina propria | Chronic inflammation with scattered clusters of eosinophils; scattered strands of smooth muscles | Lymphoid aggregates and mixed inflammation; scattered strands of smooth muscles; edematous | Mixed inflammation with numerous small congested vessels; edematous |
| Gland to stromal ratio | High | Low | Low |

in exon 11 and a deletion of four base pairs in exon 9 at codon 415.[17] Histologically, gastric polyps in JPS are similar to other hyperplastic or hamartomatous polyps and are characterized by foveolar hyperplasia. Compared to MD, gastric juvenile polyps show distorted glandular architecture, edematous stroma, lower gland to stroma ratio, and less conspicuous eosinophils and smooth muscle fibers in the lamina propria (Table 2, Fig. 2B, E). Among 48 patients referred to Vanderbilt University Medical Center with a diagnosis of MD for a clinical trial of cetuximab conducted in individuals with advanced MD, 25 (52%) were confirmed with the diagnosis of MD. The most common entities mistaken for MD were gastric hyperplastic polyps or JPS, found in seven of the total 23 non-MD patients (30%).[8]

Gastric polyps in syndromes with both gastrointestinal and extra-intestinal manifestations can also mimic MD; therefore, clinical information is essential for a correct diagnosis. Cronkhite-Canada syndrome (CCS) is a rare nonhereditary syndrome characterized by diffuse polyposis in the gastrointestinal tract, alopecia, cutaneous hyperpigmentation, and onychodystrophy.[18] Gastric polyps of CCS share many histologic features with juvenile polyps. Mixed inflammation in the lamina propria with prominent eosinophils and eosinophilic infiltration of glandular epithelium with crypt abscesses is a helpful upper gastrointestinal feature of CCS. Peutz-Jeghers syndrome (PJS) is characterized by hamartomatous gastrointestinal polyps with increased skin and mucosal pigmentation. PJS is associated with mutations in the serine/threonine kinase 11 (*STK11*) gene and shows an autosomal dominant inheritance pattern. PJS polyps are usually characterized by hyperplastic epithelium divided by arborizing bands of smooth muscle. However, PJS polyps in the stomach show dilated hyperplastic glands and less prominent smooth muscle strands compared to PJS polyps in other gastrointestinal tract (Fig. 2C, F).[19] This makes it challenging to differentiate them from hyperplastic polyps or MD. Cowden syndrome is characterized by numerous hamartomas in the gastrointestinal tract and extraintestinal sites such as skin, breast, thyroid, gynecologic, oral cavity, and central nervous system. Mutations in the phosphatase and tensin homolog (*PTEN*) gene are detected in up to 80% of Cowden syndrome cases and show an autosomal dominant inheritance pattern.[20] A diffuse "carpetlike" distribution of white mucosal plaques in the esophagus is a characteristic endoscopic finding, which microscopically shows thickened squamous epithelium with glycogen accumulation in the cytoplasm (glycogenic acanthosis).[21] Histologic features of Cowden syndrome overlap those of hyperplastic polyps or juvenile polyps.

Gastric adenocarcinoma and proximal polyposis of the stomach is a recently described entity with an autosomal dominant inheritance with incomplete penetrance. The genetic cause of this disorder is unknown. Numerous gastric polyps carpeting the proximal stomach are observed endoscopically with sparing of the esophagus, antrum, duodenum, and colon. These polyps histologically overlap with fundic gland polyps with multiple foci of low- and high-grade dysplasia. Occasional hyperplastic or adenomatous polyps can occur. This syndrome is associated with a high risk of intestinal-type gastric adenocarcinoma, and 41 cases in five families have been reported to date. Nine of the cases developed gastric adenocarcinoma.[22,23] Other entities that can mimic hypertrophic gastropathy include diffuse gastric carcinoma, gastric lymphoma, and amyloidosis.

## TRANSFORMING GROWTH FACTOR ALPHA IN MÉNÉTRIER'S DISEASE

The pathophysiology of MD has not been fully elucidated. However, the observation that transgenic mice overexpressing transforming growth factor alpha (TGF-α) in the stomach develop gastric changes that resemble MD and increased TGF-α expression in the gastric mucosa of MD patients suggest an important role of this growth factor in its pathogenesis.[24-31]

TGF-α is one of seven mammalian ligands that bind epider-

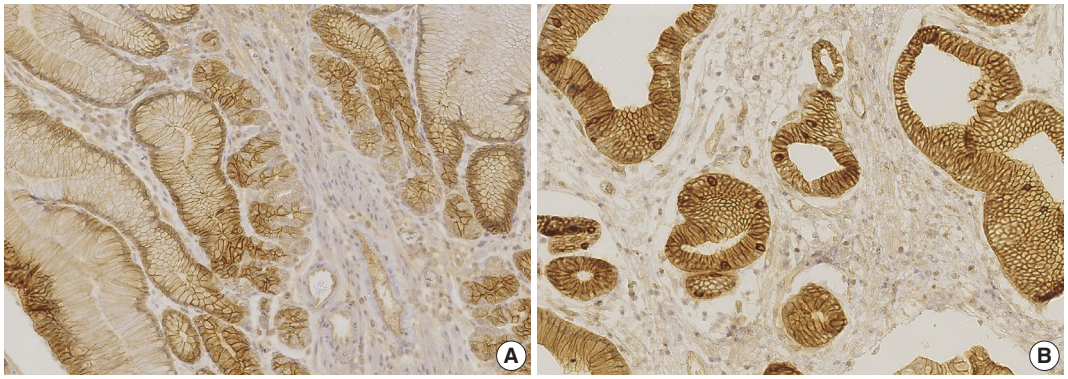


**Fig. 3.** Immunohistochemistry of phosphorylated EGFR (pEGFR). Hyperplastic foveolar epithelium of both Ménétrier's disease (A) and juvenile polyp (B) shows membranous pEGFR staining.

mal growth factor receptor (EGFR).[32] EGFR is a receptor tyrosine kinase that mediates signal transduction in many cell types. A common action of TGF-α is increased cellular proliferation, and elevated TGF-α expression has been associated with neoplastic transformation.[33,34] Transgenic mouse models that overexpress TGF-α showed epithelial hyperplasia of liver, pancreas, stomach, small intestine, colon, mammary glands, and coagulation glands. Acinoductular metaplasia was also found in the pancreas, and neoplasia was identified in the mammary glands and liver.[25,28,31] Interestingly, the stomachs of mice that overexpressed TGF-α showed characteristic MD features, such as foveolar hyperplasia, glandular cystic dilatation, oxyntic atrophy, increased mucin production, and reduced acid secretion.[24,26,27] Moreover, it has been shown that TGF-α expression in both RNA and protein is increased in the gastric mucosa of MD patients.[24,29,30] Functionally, TGF-α overexpression appears to direct gastric stem/progenitor cells to surface mucous cell differentiation at the expense of parietal and chief cell differentiation.[35-37] Additionally, TGF-α overexpression repatterns gastric fundic-type epithelium into antral-type epithelium, as evidenced by aberrant expression of the gastric antral markers, Pdx1 and gastrin, in the gastric body of both TGF-α transgenic mice and MD patients.[38]

Involvement of EGFR signaling in the pathogenesis of MD is further supported by a CMV-associated variant of MD. It has been shown that the CMV principal envelope glycoprotein, gB, binds EGFR, induces EGFR-HER3 hetero-oligomers, and activates EGFR signaling.[39] In addition, disruption of the bone morphogenetic protein/TGF-β signaling pathway in mice can lead to increased levels of TGF-α and amphiregulin, another EGFR ligand.[40-42] We also found that phosphorylated EGFR is increased in the involved gastric mucosa of JPS patients (Fig. 3). These findings could contribute to the histolopathologic similarities between gastric JPS and MD.

## TREATMENT

It is advised to rule out spontaneous remission variants associated with CMV or *Helicobacter pylori* infection by appropriate clinical testing. CMV-associated MD usually resolves within several weeks to months.[4,43] *H. pylori* eradication can be attempted if the organism is detected. Other than these approaches, there is currently no standard medical treatment for MD. Reports on efficacy of steroids, anticholinergics, acid suppression, and octreotide are inconsistent.[8] Partial or total gastrectomy is reserved for patients with intractable or debilitating disease and for cases with a high risk of developing gastric cancer. The link to TGF-α and EGFR signaling in the pathogenesis of MD led us to design a clinical trial using a neutralizing monoclonal antibody (cetuximab) against EGFR for MD patients. All seven patients who completed a month of cetuximab treatment showed statistically significant improvement in quality-of-life indices, increased parietal cell mass and gastric acidity. In addition, four patients showed near-complete histological resolution. To our surprise, improvement in symptoms usually started within one to two days after the first cetuximab infusion, and parietal cell mass was significantly increased within 24 hours.[11] Therefore, based on the results of this study, cetuximab should be considered a therapeutic option for MD patients.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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REVIEW

# Idiopathic Noncirrhotic Portal Hypertension: An Appraisal

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Idiopathic noncirrhotic portal hypertension is a poorly defined clinical condition of unknown etiology. Patients present with signs and symptoms of portal hypertension without evidence of cirrhosis. The disease course appears to be indolent and benign with an overall better outcome than cirrhosis, as long as the complications of portal hypertension are properly managed. This condition has been recognized in different parts of the world in diverse ethnic groups with variable risk factors, resulting in numerous terminologies and lack of standardized diagnostic criteria. Therefore, although the diagnosis of idiopathic noncirrhotic portal hypertension requires clinical exclusion of other conditions that can cause portal hypertension and histopathologic confirmation, this entity is under-recognized clinically as well as pathologically. Recent studies have demonstrated that variable histopathologic entities with different terms likely represent a histologic spectrum of a single entity of which obliterative portal venopathy might be an underlying pathogenesis. This perception calls for standardization of the nomenclature and formulation of widely accepted diagnostic criteria, which will facilitate easier recognition of this disorder and will highlight awareness of this entity.

**Key Words:** Idiopathic; Noncirrhotic; Hypertension, portal; Liver; Histopathology

Portal hypertension is a manifestation of increased resistance to portal venous flow due to prehepatic, intrahepatic, or posthepatic causes. The intrahepatic causes are further divided into presinusoidal, sinusoidal, and postsinusoidal causes. The two most common causes of portal hypertension are cirrhosis and schistosomiasis,[1,2] both of which are intrahepatic. When no definitive cause for portal hypertension is identified in the absence of cirrhosis, the condition is referred as an idiopathic noncirrhotic portal hypertension (INCPH).[3]

Although INCPH has been recognized for more than one century, the knowledge about this disorder is relatively limited due to clinical and pathologic under-recognition and lack of standardized nomenclature and diagnostic criteria. Recent morphologic studies have greatly enhanced our understanding about INCPH by demonstrating its wide histologic spectrum and its common associations with other systemic conditions. Moreover, these studies have revealed that diverse morphologic entities of different names, such as hepatoportal sclerosis (HPS), partial nodular transformation (PNT), incomplete septal cirrhosis (ISC), and nodular regenerative hyperplasia (NRH), indeed represent temporal and spatial heterogeneity of a single condition whose underlying pathogenesis is obliterative portal venopathy (OPV).[4-7]

Recently, Schouten *et al*.[8] proposed INCPH as a unifying diagnostic terminology in order to encompass the variable histopathologic entities manifesting as portal hypertension in the absence of cirrhosis, with a clinical connotation in the terminology. Standardization of the nomenclature will not only promote optimal patient care, but will also facilitate sharing of knowledge and collaborative research, which will lead to development of guidelines for treatment and diagnostic criteria. This paper summarizes the historical background of INCPH including the evolution of the terminology and provides an updated review of its clinical and pathological aspects.

## DEFINITION AND DIAGNOSIS

The Asian Pacific Association for the Study of the Liver (APASL) defines INCPH (referred as noncirrhotic portal fibrosis [NCPF]/idiopathic portal hypertension [IPH]) as "a disease of uncertain etiology characterized by a periportal fibrosis and involvement of small and medium branches of the portal vein, resulting in the development of portal hypertension."[9] In Western countries, the condition is poorly characterized and does not have a widely accepted definition, possibly due to its rarity. Generally, it is regarded as a clinical entity of intrahepatic portal hypertension with no evidence of cirrhosis, other liver diseases that might be ac-

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countable for portal hypertension, and splanchnic venous thrombosis.[3,6,10,11]

The diagnosis of INCPH is rendered in patients with portal hypertension after excluding portal vein thrombosis, Budd-Chiari syndrome, exposure to medications or toxins, splenic vein thrombosis, and other liver disease that can manifest as portal hypertension, followed by a confirmatory liver biopsy.[3] Since INCPH is a diagnosis of exclusion, this poses challenges to clinicians and pathologists, and many patients carry a presumed diagnosis of cirrhosis.[3,12]

## HISTORY AND NOMENCLATURE

Between 1884 and 1910, Banti[13] described patients with splenomegaly and anemia without hematologic disorders. He speculated that the natural history of this disorder consisted of three phases—initial phase of splenomegaly and anemia, followed by a transitional phase, and finally progressing to a terminal phase of gastrointestinal hemorrhage, liver failure, and death.[14] In retrospect, his cohort was heterogeneous and included patients with cirrhosis, tropical splenomegaly due to malaria, and INCPH.[8] This "Banti's syndrome" was thought to represent a primary splenic disorder with secondary changes in the liver, with endophlebitis as a common pathogenesis.[15]

The paradigm shift occurred in 1934, when McMichael[16] attributed the pathologic changes of the portal veins to portal hypertension, in patients with "hepatolienal fibrosis," i.e., splenomegaly without liver cirrhosis. In 1936, the Spleen Clinic at Columbia Presbyterian Hospital in New York reported 15 patients with splenomegaly with no evidence of cirrhosis or obstruction of the portal venous system.[17] Subsequently, in 1945, Whipple[18] reported that 26 of 93 patients with splenomegaly were without cirrhosis, schistosomiasis, or extrahepatic portal vein obstruction. The term "hepatoportal sclerosis (HPS)" was coined in 1965 by Mikkelsen *et al*.[19] for this condition. In their paper, the authors documented histologic evidence of phlebosclerosis of the intrahepatic and extrahepatic branches of the portal vein in 36 patients with noncirrhotic portal hypertension.[19] Phlebosclerosis was recognized as partial or complete obliteration of the portal vein lumen. Comparable histologic observation was reported in a study from Calcutta, India, wherein the authors used the term "idiopathic portal hypertension (IPH)" in the title and "noncirrhotic portal fibrosis (NCPF)" in the text in order to designate portal hypertension without cirrhosis or extrahepatic portal obstruction.[20] In addition, this study demonstrated a better prognosis of NCPF/IPH compared to that of cirrhosis. The term NCPF was subsequently endorsed by the Indian Council of Medical Research.

The pathogenic terminology "obliterative portal venopathy (OPV)" was introduced by Nayak and Ramalingaswami[21] in their pathologic study of noncirrhotic portal hypertension. OPV was characterized by segmental, conspicuous subendothelial thickening of large- and medium-sized intrahepatic portal vein branches. In addition, scarring and obliteration of small portal vein branches along with an increased number of small vascular channels within the portal tracts and incomplete thin fibrous septa were noted.[21] In Japan, the term IPH was used in a national survey performed by the Ministry of Health and Welfare.[22] Other names given to this disorder include noncirrhotic intrahepatic portal hypertension, benign intrahepatic portal hypertension, and idiopathic presinusoidal portal hypertension.[6,14,23,24] To date, the names OPV and HPS have been commonly used in the Western literature, and IPH and NCPF have been widely used in the Eastern regions. The unifying term INCPH was proposed in a review paper by Shouten *et al*,[8] since this nomenclature addresses both clinical and histopathological aspects of the entity.

## ASSOCIATED HISTOPATHOLOGIC ENTITIES OF IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION

NRH of the liver was first described by Ranstrom in 1953,[25] in a patient with Felty's syndrome, and was called "miliary hepatocellular adenomatosis." The term NRH was used by Steiner in 1959[26] as a descriptive histopathologic term to be distinguished from cirrhosis. Subsequently, case reports and case series have revealed that a subset of patients with NRH present with portal hypertension.[7] This association was confirmed in a large-scale autopsy study, wherein 2.6% of cases had NRH, and 4.7% of these were found to have evidence of portal hypertension. Moreover, all NRH cases showed obliterative changes of the portal veins.[27]

ISC was described by Popper in 1966[28] as a subtype of macronodular cirrhosis, wherein inconspicuous, large regenerative nodules are vaguely delineated by thin and frequently incomplete septa. Some authors postulated that ISC represents regressed cirrhosis.[29]

PNT of the liver was described by Sherlock *et al*. in 1966,[30] in four cases of portal hypertension, three of which were from autopsy. In PNT, the liver parenchyma adjacent to the hilum shows macroscopic and microscopic nodular transformation without advanced fibrosis, while the periphery is either atrophic or

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normal. The authors coined the term PNT to avoid confusion with other nodular lesions of the liver, such as cirrhosis (diffuse nodular transformation with extensive fibrosis), focal nodular hyperplasia (focal nodularity usually in the periphery, without portal hypertension), and NRH (diffuse nodular transformation without significant fibrosis). Histologic features of OPV were not evaluated in this original study; however, subsequent morphometric study suggested that portal vein obliteration might be involved in the pathogenesis of PNT.[31]

Although these entities were initially reported as distinct disorders, subsequent morphologic studies demonstrated that NRH, ISC, and PNT had overlapping clinical and pathologic features with INCPH, supporting that these histopathologic entities share a common etiopathogenesis and most likely represent part of the histologic spectrum of a single condition.[4-7]

## EPIDEMIOLOGY

INCPH is commonly reported in developing countries and in lower socioeconomic groups.[32,33] INCPH is a common cause of portal hypertension in Japan and the Indian Subcontinent, constituting up to 30% and 40% of the cases, respectively, while only 3%–5% of portal hypertension in Western countries is attributed to INCPH.[32,34,35] However, the true prevalence of INCPH might be higher since patients are frequently misdiagnosed as having cirrhosis. Additionally, a significant proportion of such patients is in the subclinical phase of INCPH and might go unrecognized.[3,12,27]

A male predilection has been reported in India and the West, whereas INCPH is more common in women in Japan.[36-40] The age of onset of INCPH tends to be younger in patients from India (25–35 years) compared to Japan (43–56 years).[36-40] Limited data from the West have shown that the median age of onset is about 40 years.[8] INCPH has also been reported in children.[41-45]

## ETIOLOGY

No definite etiology is identified in more than half of the patients with INCPH.[46] Nevertheless, INCPH has been frequently reported in a multitude of immunologic disorders, including systemic lupus erythematosus, myasthenia gravis, systemic sclerosis, celiac disease, thyroiditis, rheumatoid arthritis, Crohn's disease, Felty's syndrome, Sjogren's syndrome, autoimmune hepatitis, primary biliary cirrhosis, common variable immunodeficiency, and hypergammaglobulinemia, raising the possibility of an immunologic cause as an underlying etiology.[8,25,46-55] A survey from Japan showed that about 70% of female patients with INCPH had anti-DNA antibodies, and 24% and 21.5% showed antinuclear antibodies and antimicrosomal antibodies, respectively.[2,56] Likewise, an increased incidence of immune complex-associated glomerulonephritis was reported in INCPH patients following spleno-renal shunt, compared to those with normal liver.[57]

Higher prevalence of INCPH in lower socioeconomic groups and experimental animal studies indicate an infectious etiology.[3,52,58,59] Especially in the West, INCPH is increasingly recognized in human immunodeficiency virus (HIV) patients.[3,32] Earlier studies have postulated that the use of didanosine, an antiviral medication of the reverse transcriptase inhibitor class with a potential for mitochondrial toxicity, might be associated with development of INCPH.[60,61] However, a recent multicenter case-control study showed that some of these patients were genetically predisposed to develop this condition.[62] In that study, a subset of HIV patients with prior exposure to didanosine and who subsequently developed INCPH was found to be associated with higher frequency of four specific single-nucleotide polymorphisms (SNPs) at the two genes coding enzymes of purine metabolism.[62] Moreover, the cumulative risk of developing INCPH was postulated to be 100% in the presence of all four SNPs. Alternatively, direct virus-induced sinusoidal endothelial cell injury might lead to INCPH in HIV patients.[63] Therefore, it is difficult to identify the precise cause of INCPH in HIV patients.

In addition to didanosine in HIV patients, exposure to various medications, chemicals, and toxins has been reported to be associated with INCPH.[3,8,32,36] For example, history of pica was noted in 46% of INCPH patients in Iran,[64] and radiation and chemotherapy have been reported to result in INCPH.[65,66]

Occurrence of INCPH in patients with congenital disorders including Adams-Oliver syndrome, Turner syndrome, phosphomannose isomerase deficiency, and familial cases of INCPH indicate a certain genetic makeup in these patients,[3,45,67-72] making them susceptible to INCPH. In a report of four families with INCPH, six of seven members (85.5%) with INCPH were shown to be HLA-DR3 positive.[72]

Lastly, the association between hypercoagulability and INCPH is relatively well established. Up to 54% of INCPH patients have been reported to be thrombophilic,[6,32,73] with secondary portal vein thrombosis being relatively common. In addition, some of the characteristic histologic features of INCPH, such as obliteration and muscular hypertrophy of portal venous branches, might be explained by a prior/persistent thromboembolic event.[3] Thrombotic changes have also been noted within the portal veins and their larger branches in autopsies of INCPH patients.[4] In

HIV patients, acquired protein S deficiency with resultant thrombophilia and INCPH has been reported,[74] suggesting a role of hypercoagulability in these patients. Likewise, liver biopsies from patients with primary portal vein thrombosis without cirrhosis frequently show phlebosclerosis and NRH.[66,75] Given the association between INCPH and hypercoagulability, anticoagulation has been advocated as a potential treatment option for INCPH.[6]

## CLINICAL PRESENTATION

Patients with INCPH usually present with signs and symptoms associated with complications of portal hypertension, including upper gastrointestinal variceal bleeding, splenomegaly, and hypersplenism (anemia, thrombocytopenia, and leukopenia). The hepatic venous pressure gradient, the difference between wedged and free hepatic venous pressures, is significantly lower in INCPH than in cirrhosis and might be normal or only mildly elevated. In contrast, portal venous pressure is markedly elevated. These findings are indicative of presinusoidal portal hypertension.[32,58,76] Anorectal varices are also common in INCPH, but bleeding from anorectal varices is uncommon.[77] Ascites, encephalopathy, hepatorenal syndrome, and jaundice can occur with a lower frequency, and some patients present with extrahepatic portal vein thrombosis.[3,12,32,78] Liver enzymes can be normal or slightly abnormal; presentation with isolated liver enzyme abnormalities was previously reported in 20% of INCPH cases.[46] Hepatic synthetic function is mostly preserved but can be rarely compromised, requiring liver transplantation.[79]

## IMAGING STUDIES

Ultrasonography might show nodularity of the liver surface and thickened portal venous wall.[4,78,80] Computed tomography and magnetic resonance imaging can show signs of portal hypertension, extrahepatic portal vein thrombosis, intrahepatic portal abnormalities, nodular liver contour, and hypertrophy of the caudate lobe with atrophy of segment IV. The latter two features are more commonly seen in cirrhosis.[70,81] When measured using elastography, the mean liver stiffness in INCPH (8.4 ± 3.3 kPa by transient elastography and 1.56 [0.98–2.37] m/sec by acoustic radiation force impulse elastography) is lower than that of cirrhosis (40.9 ± 20.5 kPa and 2.44 [1.08–3.83] m/sec, respectively).[76,82]

## PATHOLOGIC FEATURES

The gross appearance of the liver is heterogeneous and can be normal, enlarged, or atrophic with a smooth, wrinkled, or nodular surface (Fig. 1).[9,21,32] In patients requiring liver transplantation for advanced INCPH, the explanted liver tends to be atrophic with frequent surface nodularity.[12,79] Subcapsular septation, prominence of portal tracts near the surface, and sclerosis of portal vein branches with or without organized thrombi have been described.[9,21,40,83] Relative hypertrophy of the right lobe and atrophy of the left lobe are common findings.[21] The cut surface might be slightly nodular or partially nodular near the hepatic hilum.[21,79]

The histopathological features of INCPH vary and are dependent on the phase of the disease as well as the area sampled.[40] Variable combinations of these histological components such as OPV, variable portal fibrosis, vascular abnormalities, and NRH can be seen. The hallmark of INCPH is OPV, characterized by dense fibrosis/sclerosis of the portal vein along with portal/periportal fibrosis, phlebosclerosis of portal vein branches with resultant decrease of the lumen (Fig. 2), an increase in the number of portal vascular channels (Fig. 3), and arterialization of the portal vein branches. In addition, there can be portal shunting vessels that directly connect periportal areas with the hepatic

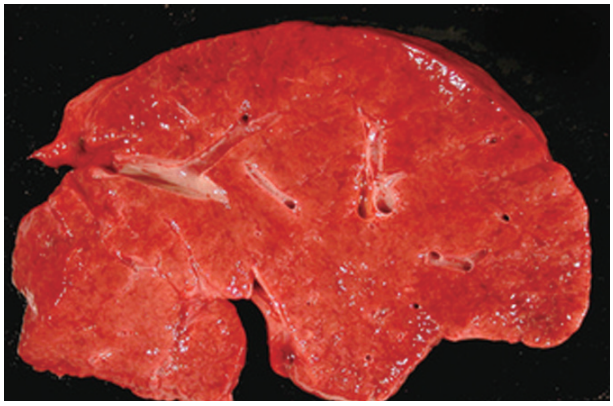

**Fig. 1.** Gross cut surface of a liver with idiopathic noncirrhotic portal hypertension demonstrates vague nodularity without cirrhosis.

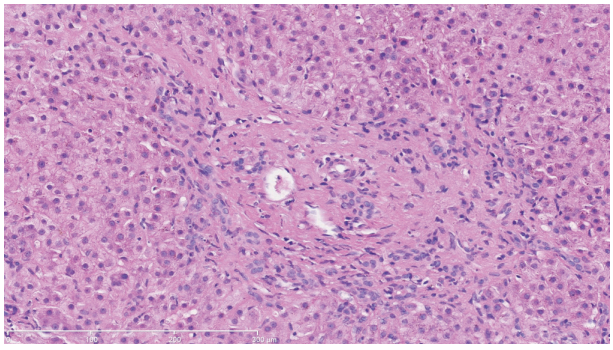

**Fig. 2.** Phlebosclerosis with narrowed venous lumen.


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lobule (Fig. 4). Mild lymphocytic portal inflammation and mild bile ductular proliferation might be seen.[11,12,21,66] Changes in the lobules include diffuse or focal nodular regeneration (Fig. 5), dilated sinusoids (megasinusoids) (Fig. 6), increased number of venous profiles per lobule (Fig. 7), with architectural distortion (Fig. 8), and incomplete septa, i.e., slender fibrous septa originating from a portal tract that blindly ends in the lobule, perisinusoidal fibrosis, and perivenular fibrosis.[3,6,8,11,12,32,66,79] Portal tract remnants, or rudimentary/hypoplastic portal tracts—small portal tracts wherein the lumen of the bile duct or artery is smaller than adjacent hepatocytes, with inconspicuous or sometimes absent portal vein branches—might be identified (Fig. 9). The above histologic features are consistently reported in INCPH; however, the specificity of individual histologic findings remains unclear. For example, portal fibrosis and portal venous obliteration have also been seen in control livers without INCPH.[4] Similarly, the histologic features of INCPH were seen in patients with multiple comorbidities, without established diagnosis of INCPH.[11]

Verheij *et al*.[66] evaluated variable histologic features of INCPH in Western patients and found that portal tract remnants, phlebosclerosis of portal vein branches, and NRH are more common in INCPH compared to noncirrhotic portal vein thrombo-

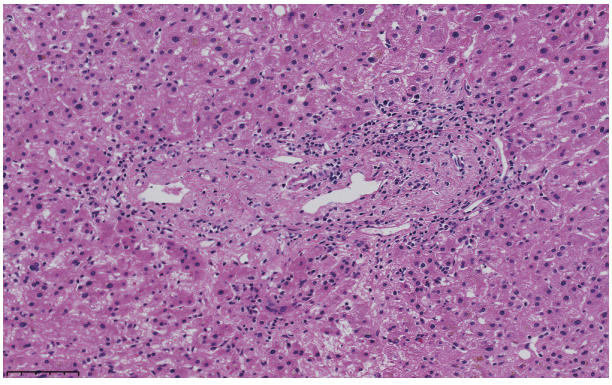

**Fig. 3.** Portal sclerosis associated with increased number of vascular channels.

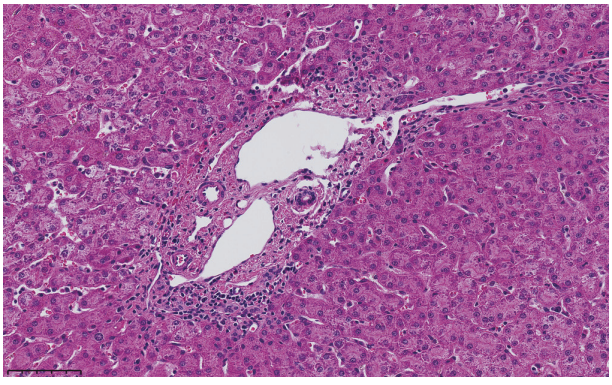

**Fig. 4.** Abnormally dilated portal venous branches associated with herniation of the vein into the hepatic lobule (shunt vessel).

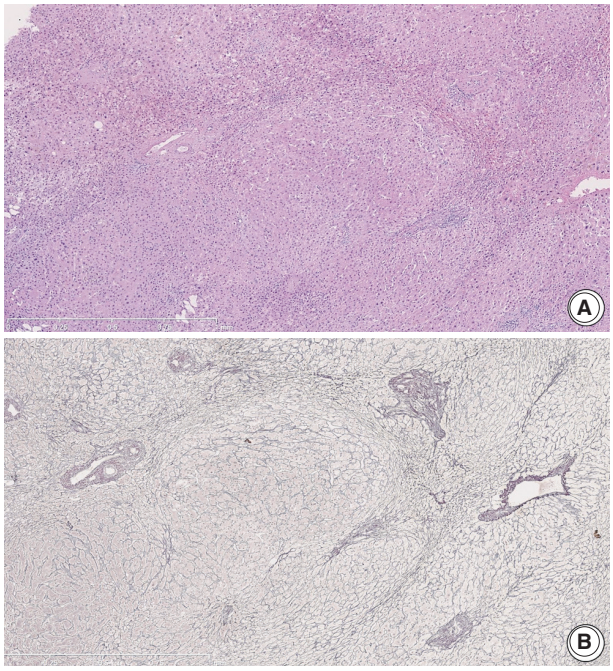


**Fig. 5.** (A) Regenerative nodules of the lobule without cirrhosis. (B) Reticulin stain of the corresponding area highlights regenerative nodules.

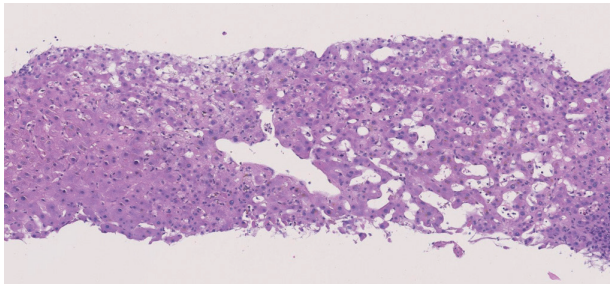

**Fig. 6.** Dilated sinusoids (also known as megasinusoids).

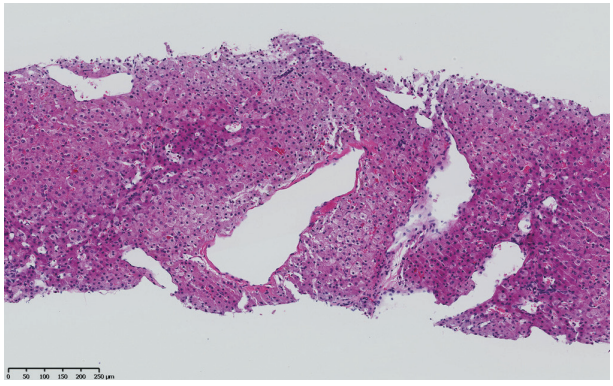

**Fig. 7.** Abnormally dilated veins in the lobule.

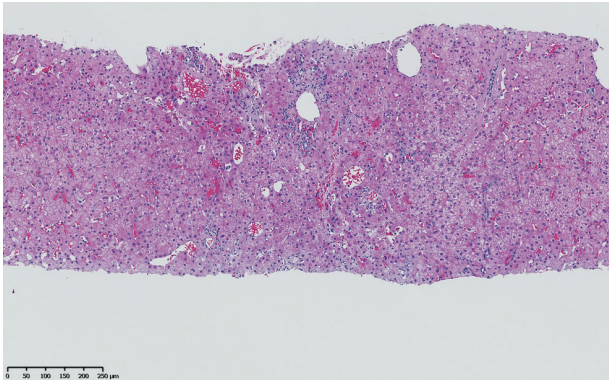

**Fig. 8.** Distorted hepatic architecture in idiopathic noncirrhotic portal hypertension.

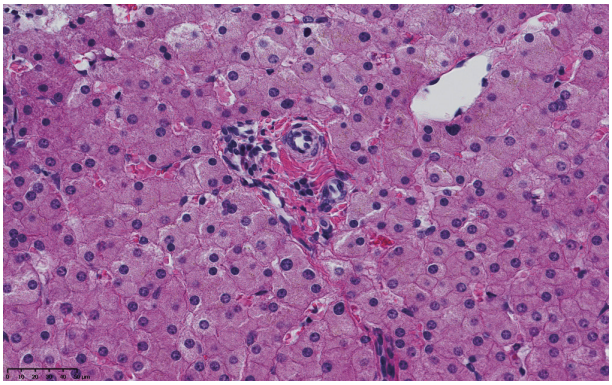

**Fig. 9.** Hypoplastic/rudimentary portal tract.

sis. In contrast, sinusoidal dilatation, shunting vessels, increased number of portal vessels, and increased number of veins per lobule were suggested to represent secondary changes of portal hypertension.[66] In addition, NRH was significantly more common in HIV-associated INCPH, whereas portal tract remnants were frequent in INCPH without HIV. Nevertheless, no correlation has been found between the histomorphology and clinical signs of portal hypertension.[66]

## TREATMENT AND OUTCOME

The treatment primarily consists of controlling and preventing the symptoms of portal hypertension, especially variceal bleeding. The management strategy used for cirrhotic patients with portal hypertension is currently being used for INCPH, with a favorable long-term outcome.[10] For example, acute hemorrhage from esophageal varices is treated with combined vasoactive drugs and endoscopic variceal ligation/sclerotherapy. Transjugular intrahepatic porto systemic shunting (TIPS) can be offered to patients who fail to respond to endoscopic therapy or those with recurrent bleeding. Prophylaxis for variceal bleeding consists of the use of non-selective beta blockers, endoscopic variceal ligation, or TIPS in selected patients.[3,84] In addition, any drugs associated with development of INCPH are discontinued, and medical conditions associated with INCPH should be treated.[3]

Preliminary data suggests that anticoagulation for thrombophilic INCPH patients might be beneficial. In a case series, eight of 15 INCPH patients with complete or partial portal vein thrombosis responded to anticoagulation with some degree of recanalization.[10] Also, early anticoagulation for INCPH patients with hypercoagulability appeared to show a favorable clinical outcome (no death or liver transplantation),[46] and anticoagulation therapy improved liver function tests in a patient with HIV and INCPH.[85]

A small number of INCPH patients have undergone liver transplantation for complicated portal hypertension. Most of these patients carried a presumed diagnosis of cirrhosis prior to transplant.[12,79] Two patients developed histologic features of INCPH in the allograft biopsies within 1 year of the transplantation, and one of them subsequently developed recurrent portal hypertension.[12]

Although variceal bleeding is common, the overall long-term prognosis of INCPH appears to be better than that of cirrhosis, possibly due to preserved hepatic function in a majority of the patients.[3,10,20,46]

## CONCLUSION

INCPH is a rare condition that has been under-recognized both clinically and pathologically. Many different terms have been used to describe this entity, adding to the confusion. Although its management primarily focuses on the control and prophylaxis of complications of portal hypertension, the etiopathogenesis and natural history of INCPH appear distinct from those of cirrhosis. Recognition of the clinical presentation, histopathology, and associated risk factors of INCPH will enable the correct classification of patients with INCPH.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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■ REVIEW ■

# Overview of IgG4-Related Tubulointerstitial Nephritis and Its Mimickers

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Tubulointerstitial nephritis (TIN) is the most common form of renal involvement in IgG4-related disease. It is characterized by a dominant infiltrate of IgG4-positive plasma cells in the interstitium and storiform fibrosis. Demonstration of IgG4-positive plasma cells is essential for diagnosis, but the number of IgG4-positive cells and the ratio of IgG4-positive/IgG-positive plasma cells may vary from case to case and depending on the methods of tissue sampling even in the same case. IgG4-positive plasma cells can be seen in TIN associated with systemic lupus erythematosus, Sjögren syndrome, or anti-neutrophil cytoplasmic antibody–associated vasculitis, which further add diagnostic confusion and difficulties. To have a more clear view of IgG4-TIN and to delineate differential points from other TIN with IgG4-positive plasma cell infiltrates, clinical and histological features of IgG4-TIN and its mimickers were reviewed. In the rear part, cases suggesting overlap of IgG4-TIN and its mimickers and glomerulonephritis associated with IgG4-TIN were briefly described.

**Key Words:** IgG4-related disease; Lupus nephritis; Sjögren's syndrome; Anti-neutrophil cytoplasmic antibody-associated vasculitis; Glomerulonephritis, membranous

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory disorder involving almost any organ in the body.[1-3] Tubulointerstitial nephritis (TIN) is the most common form of renal involvement, which characterizes a dominant interstitial infiltrate of IgG4-positive plasma cells and storiform fibrosis.[1] Although TIN showing similar histologic features have been reported previously,[4] a connection with IgG4-RD demonstrating IgG4-positive cells in the interstitium was first reported in 2004.[5,6] Since then, case studies and collective reviews on TIN with dominant IgG4-positive cell infiltrate (IgG4-TIN) have been rapidly cumulated during the next 10 years.[7,8] Presently, we have more comprehensive understanding on renal manifestations of this systemic disease, but at the same time, we have come to recognize cases showing variable histology and wide clinical spectrum, some of which do not fit into the narrow spectrum of IgG4-TIN.

IgG4 is unique as it does not activate complements. The role of IgG4 in inflammation and immune deposits has not been clarified yet. Nonetheless, the presence of IgG4-positive plasma cells is a characteristic feature of IgG4-TIN as the name is adopted, and immune deposits may be observed in some cases. The degree of IgG4-positive cell infiltrate and its ratio among the infiltrating cells may vary from case to case and they depend on the sampling methods even in the same case. IgG4-positive plasma cells may be seen in other diseases and may be numerous in some cases of autoimmune diseases.[9] Furthermore, clinical and laboratory features characteristic of IgG4-TIN may be present in TIN of systemic lupus erythematosus (SLE), Sjögren syndrome, or anti-neutrophil cytoplasmic antibody (ANCA)–associated vasculitis. It is important to distinguish TIN cases because therapeutic plans and prognosis may differ depending on the causes.

To have an overview of TIN with IgG4-positive plasma cell infiltrates and to delineate hints for differential diagnosis, clinical and histological features of IgG4-TIN and its mimickers are reviewed. In the rear part, atypical TIN cases showing clinical and laboratory overlaps of IgG4-TIN and its mimickers and glomerulonephritis associated with IgG4-TIN are briefly described.

## TUBULOINTERSTITIAL NEPHRITIS IN IMMUNOGLOBULIN G4-RELATED DISEASE

Renal histology is fundamental in the diagnosis of TIN in IgG4-RD. Three features are characteristic: (1) interstitial lymphoplasmacytic infiltrates with dominant IgG4-positive plasma cells; (2) the ratio of IgG4-positive/IgG-positive plasma cells over 40%; and (3) obliterative phlebitis. A cut-off value of > 10 IgG4-positive plasma cells/high-power field (HPF) and/or ratio of IgG4-positive/IgG-positive plasma cells > 40% was used in





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the previous Japanese study.[10] Soon after, in the consensus guideline on IgG4-RD in 2012,[11] different cut-off values were applied in the number of IgG4-positive plasma cells according to the type of specimen received. In renal biopsy samples, > 10 IgG4-positive plasma cells/HPF are enough, but > 30 IgG4-positive plasma cells/HPF are required in nephrectomy specimens. The infiltrate may be patchy in distribution; therefore, the possibility of IgG4-RD should not be excluded based on negative biopsy results, especially in the presence of other supportive clinical and imaging features of IgG4-RD. The IgG4/IgG ratio of plasmacytic infiltration over 40% was maintained in the consensus guideline, which is a reasonable value as it demonstrated a sensitivity of 58.8% and a specificity of 90.2% in a meta-analysis.[12] In the lymphocytic infiltrates, T lymphocytes predominate over B cells. Eosinophils are common and may be numerous in some cases. Inflammatory infiltrates may extend into the renal capsule, which has not been known in TIN of other non-infectious causes[13,14] (Fig. 1A–D). Glomeruli are usually spared, but when

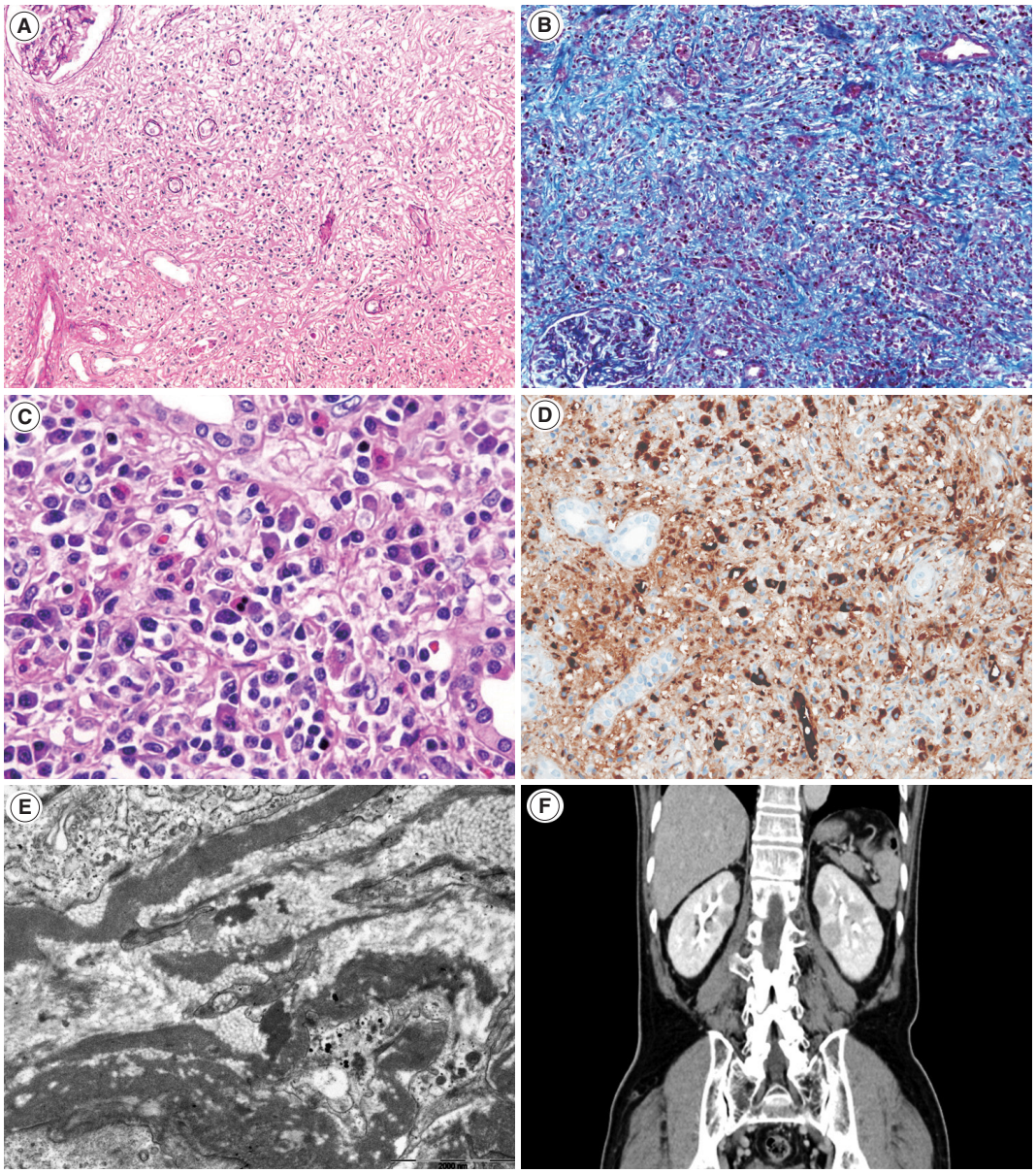


**Fig. 1.** Tubulointerstitial nephritis in IgG4-related disease. (A, B) At lower power, interstitial fibrosis is severe and shows a focal streaming pattern with mixed inflammatory infiltration of lymphocytes and plasma cells (A, periodic-acid Schiff ×100; B, Masson trichrome ×100). (C) In some cases, eosinophil infiltration may be prominent (hematoxylin-eosin. ×400). (D) Many IgG4-positive plasma cells are present in the interstitium (IgG4, ×200). (E) On electron microscopy, fine granular electron-dense deposits are present in the interstitium (×15,000). (F) Contrast-enhanced computed tomography shows patchy multiple round or wedge-shaped parenchymal low-density lesions in both kidneys.

glomerulonephritis is associated, membranous nephropathy is the most common.[15] Vascular changes are not common, but renal arteritis was reported in one case associated with TIN.[16]

Tubulointerstitial immune deposits may be seen in some cases.[17] IgG and C3 are deposited most commonly along the tubular basement membrane.[18] Interstitial immune deposits tend to be restricted in inflamed areas and they are regarded as a late change.[18] By electron microscopy, electron-dense deposits are frequently found in the tubular basement membrane and interstitium[13] (Fig. 1E). In an earlier report of idiopathic hypocomplementemic TIN, which is now regarded as a form of IgG4-TIN, fingerprint organoid deposits were present in the interstitium in two out of nine cases.[4] Glomerular mesangial and Bowman's capsular deposits were also frequently observed under electron microscopy, even though glomeruli showed no significant changes by light and immunofluorescent microscopy.[19]

Most IgG4-TIN patients are males in mid-sixties. Renal involvement was reported in six of 132 (4.5%),[20] 10 of 114 (8.8%),[21] 54 of 235 (23.0%),[22] and 20 of 57 (35.1%) patients[23] with IgG4-RD. Patients present with acute renal failure, urinary abnormalities, or mass formation with urinary obstruction.[7,19] Urinalysis showed mild proteinuria in 82.6% and hematuria in 34.8% in one collective study.[7] If membranous nephropathy is associated, nephrotic range proteinuria may be present.[15] In the presence of renal failure, serum creatinine levels may be elevated. Serology shows polyclonal hypergammaglobulinemia and elevated IgG4 levels. Serum IgG4 levels (> 135 mg/dL) may be elevated in up to 93% of IgG4-TIN,[7,8,10] which are higher than the prevalence of about 70% in total IgG4-RD. This high rate may be related to the organ specificity, but it may reflect the increased number of involved organs because other organs are frequently involved at the time of diagnosis.[22] Serum IgG4 levels have been reported to decrease with steroid treatment, and increase with relapse. However, it is neither essential nor specific for the diagnosis. Elevated IgG4 serum levels were reported in 10.8% of SLE and 12.9% of rheumatoid arthritis patients.[24] IgG4 levels may even show a paradoxical response, showing an increase despite effective treatment.[25] Other serologic markers associated with autoimmune diseases or allergies have been frequently reported. Hypocomplementemia, elevated IgE levels, and eosinophilia have been reported in 56.0%–69.6%, 71.4%, and 33.0%–47.8%, respectively.[7,8] Antinuclear antibodies (ANA) and rheumatoid factors, usually in low titers, were reported in 31.0%–69.6% and 38.9%, respectively. Anti-DNA antibody was positive in a few cases.

Although histology may be highly suggestive of IgG4-TIN, confirmatory diagnosis relies on both histological and clinical features. In addition to IgG4-positive plasma cells > 10/HPF, the presence of at least one other feature from the imaging studies, serology, or other organ involvement categories was suggested for the diagnosis.[8] Cases showing clinical and laboratory features suggestive of SLE, Sjögren syndrome, or ANCA-associated vasculitis should be excluded.[1] Other organ involvement is frequent at the time of diagnosis of TIN (83.0%–95.7%). Among them, the salivary glands (82.6%), lymph nodes (43.5%), pancreas (39.1%), and lacrimal glands (30.4%) are most frequently involved either synchronously or metachronously (Table 1).[7,8,13,26-28] In cases of renal involvement without extrarenal manifestation,[7] imaging studies may be helpful (Fig. 1F). Four patterns of round or wedge-shaped renal cortical nodules, peripheral cortical lesions, mass-like lesions, and renal pelvic involvement, were reported.[23]

IgG4-TIN responds well to steroid therapy with decrease of serum creatinine levels. A recent report of repeated biopsy after steroid treatment showed advanced fibrosis but decreased inflammatory activity with fewer IgG4-positive plasma cells and reduced expression of connective tissue growth factor mRNA.[29] Regarding disease activity, elevated serum IgG4 levels and IgG4-positive plasmablast levels were suggested in one study.[30]

## TUBULOINTERSTITIAL NEPHRITIS IN LUPUS NEPHRITIS

Lupus nephritis is usually characterized by proliferative glomerulonephritis with massive immune deposits and accompanying mild to moderate interstitial inflammation. Rarely, it may present with predominant TIN without significant glomerular changes.[31-37] Up to now, about 20 cases of predominant lupus TIN have been reported. The patients presented with acute renal failure or renal insufficiency. Interstitial inflammatory infiltrate was composed of mixture of CD4+ and CD8+ T cells, B cells, macrophages, and plasma cells. IgG, C3, and C1q deposits were present in the tubular basement membranes, whereas glomerular deposits were negative or minimal. Electron-dense deposits could be seen in tubular basement membranes and interstitium (Fig. 2).

IgG4-positive plasma cells may infiltrate in the interstitium and IgG4 deposits may be present in the peritubular interstitium and along the tubular basement membrane in lupus TIN,[36] similar to IgG4-TIN. In contrast to IgG4-TIN, immune deposits in lupus TIN are rather diffuse. C1q deposits, if prominent, favors lupus TIN. However, if mass-effects on an imaging study or patchy inflammatory infiltrate extending to renal capsule and

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**Table 1.** Review of IgG4-related tubulointerstitial nephritis

| References | No. of cases | Age (median, yr) | Male: Femlae | Extrarenal lesion | Serum IgG (median, mg/dL) | Serum IgG4 (median, mg/dL) | SCr at biopsy (mean) | Elevated Cr (> 1.2 mg/dL) | ANA (+) | RF (+) | Proteinuria (> 1 g/day) | Hematuria | Renal biopsy finding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saeki *et al.*[7] | 23 | 40–83 (64) | 20:3 | Sa (19), LN (10), Pa (9), La (7), Lu (6), Li (1), Pr (1) | 2,721–8,841 (4,387) | 305–4,630 (1,330) | 0.67–6.87 (1.98) | 13/23 | 16/23 | 7/18 | 2/23 | 8/23 | TIN (23/23), MGN (1/23), mild MPGN (3/23), focal segmental EC (1/23) |
| Raissian *et al.*[8] | 35 | 20–84 (67) | 30:5 | Sa (6), LN (8), Pa (15), La (1), Lu (8), Li (7), RP(3) | - | - | 0.9–9.0 (3.57) | 27/35 | 10/32 | - | 8/27 | 6/27 | TIN (35/35), MGN (2/35), many eosinophils (4/35) |
| Kawano *et al.*[28] | 20 | 55–83 (70) | 18:2 | Jo (1), La (2), Li (1), LN (5), Lu (6), Ne (1), Pa (7), Pr (2), RP (1), Sa (12) | 1,679–5,380 (3,596) | 408–1,860 (828) | 0.59–7.26 (1.36) | 12/20 | - | - | 2/15 | - | TIN (20/20), MPGN (1), IgAN (1), EC (2), HSPN (2), MGN (3) |
| Yamaguchi *et al.*[13] | 16 | 45–78 (62) | 12:4 | Pa (8), Sa (7), RP (1), Lu (1), Li (1) | 1,569–6,328 (3,604) | 142–2,120 (958) | 0.84–6.17 (1.6) | 12/16 | - | - | 3/11 | - | TIN (16/16), MGN (2) |

SCr, serum creatinine; Cr, creatinine; ANA, antinuclear antibodies; RF, rheumatoid factor; Sa, salivary gland; LN, lymph node; Pa, pancreas; La, lacrimal gland; Lu, lung; Li, liver; Pr, prostate; TIN, tubulointerstitial nephritis; MGN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; EC, endocapillary hypercellularity; RP, retroperitoneum; Jo, joint; Ne, nerve; IgAN, IgA nephropathy; HSPN, Henoch-Sch'onlein purpura nephritis.



**Fig. 2.** Tubulointerstitial nephritis in lupus nephritis. Granular electron-dense deposits are present in the tubular basement membrane (×3,000).

beyond are present, it is unlikely to be lupus TIN. Massive eosinophil infiltration is also exceptional for lupus.

Distinction between IgG4-RD and SLE depends on the clinical diagnostic criteria, despite some differential histologic features. The American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC) criteria may be applied to the diagnosis of SLE. Depending on the criteria applied, a variable proportion of TIN cases with IgG4-positive plasma cells may be categorized into SLE or other autoimmune diseases.[10] However, clinical distinction between IgG4-TIN and lupus TIN is not always simple. Signs of SLE may appear late and may not fulfill the diagnostic criteria at the time of biopsy.[38] Clinical and laboratory features which frequently present in IgG4-RD may also be present in SLE patients.[36] Serum gammaglobulin[35] may be elevated. Serum IgG4 levels were elevated in 10.8% of SLE patients.[24] Retroperitoneal fibrosis may be present.[39] A response to steroid treatment is also good in predominant lupus TIN.[35] Kiyama *et al.*[40] reported ANA subclasses in SLE and IgG4-RD, demonstrating IgG1, 2, or 3 subclasses in SLE and predominantly IgG2 in IgG4-RD, but very rare or no IgG4 in both conditions.

## TUBULOINTERSTITIAL NEPHRITIS IN SJÖGREN SYNDROME

Sjögren syndrome is characterized by keratoconjunctivitis sicca and xerostomia due to immunologic destruction of lacrimal and salivary glands. Renal involvement is infrequent. Distal renal tubular acidosis and acute kidney injury are the main clinical manifestations. Chronic TIN is the most commonly observed

form on renal biopsy, but glomerulonephritis may also be observed.[41,42] In TIN of Sjögren syndrome, lymphocytes with a T-cell dominance and macrophages infiltrate in the interstitium along with mild tubulitis and tubular atrophy (Fig. 3A, B). Plasma cells may be numerous but storiform fibrosis is not a feature.[43] Immune deposits may be present in the tubular basement membrane.

Clinically, symptoms of dry eye and mouth or arthralgia are more frequent in Sjögren syndrome than in IgG4-RD. The presence of anti–SS-A and anti–SS-B antibodies is characteristic and was present in 71% and 54%, respectively, in one large series.[41] Steroid therapy in Sjögren syndrome has limited effects compared with that in IgG4-RD.[44] However, clinical and laboratory features may infrequently overlap with IgG4-TIN. Serum IgG4 levels were reported to be elevated in 7.5% of the patients with primary Sjögren syndrome.[24] One TIN patient with Sjögren syndrome showed elevated serum IgG4 levels and renal interstitial IgG4-positive plasma cell infiltrate.[45] Marked hypocomplementemia was reported in a TIN case of Sjögren syndrome.[46] Some patients had "pseudolymphoma" lesions or autoimmune pancreatitis and sclerosing cholangitis.[24] By contrast, seven of 23 IgG4-TIN cases fulfilled the criteria of Sjögren syndrome,[7] and anti–SS-A antibody was present in 4.4% of Mikulicz's disease.[47]

## TUBULOINTERSTITIAL NEPHRITIS IN ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIS

Tubulointerstitial inflammation is frequently associated with ANCA-associated vasculitis. Interstitial inflammatory infiltrate is composed of lymphocytes, plasma cells, and some neutrophils. In typical cases, glomerular crescents or necrotizing lesions are commonly found with or without vasculitis and the distinction from IgG4-RD is not difficult. IgG4-positive plasma cells may be present, but the number of IgG4-positive plasma cells and/or IgG4-positive/IgG-positive plasma cell ratio is usually not high.[9] In addition, elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) is in favor of ANCA-associated vasculitis.

Rarely, IgG4-dominant TIN may present with concomitant cytoplasmic ANCA and antibody to proteinase 3.[48,49] Among the three types of ANCA-associated vasculitis, eosinophilic granulomatosis with polyangiitis (EGPA; Churg-Strauss syndrome) shows a close similarity with IgG4-RD, in terms of upper airway involvement and eosinophilia. It may be related to up-regulation of Th2 cytokines associated with increased IgG4 response.[2] Yamamoto *et al*.[50] reported elevated serum IgG4 levels and increased IgG4-positive/IgG-positive plasma cell ratio and also IgG4 renal infiltrate in EGPA patients. Vaglio *et al*.[51] showed that IgG4 levels correlated with the disease activity in EGPA patients. Chang *et al*.[52] showed increased IgG4-positive cells in 18.6% of 43 cases of granulomatosis with polyangiitis (Wegener's granulomatosis) including four kidney samples, but the cases were limited to sinonasal or orbital/periorbital biopsies. A case of ANCA-negative EGPA showed salivary gland swelling, high serum IgG4 levels, membranous nephropathy with eosinophil-rich TIN, and leukocytoclastic vasculitis.[53]

## TUBULOINTERSTITIAL NEPHRITIS IN OTHER CONDITIONS SIMULATING IMMUNOGLOBULIN G4-TUBULOINTERSTITIAL NEPHRITIS

Tubulointerstitial nephritis and uveitis with dominant IgG4-

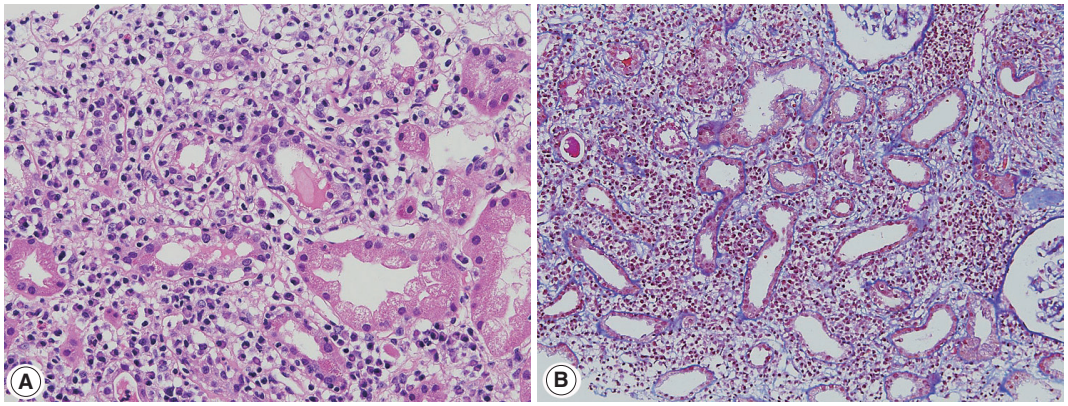


**Fig. 3.** Tubulointerstitial nephritis in Sjögren syndrome. (A) Lymphoplasmacytic infiltrate is present in the interstitium associated with moderate tubulitis (hematoxylin-eosin, ×200). (B) Interstitium is widened by edema and cellular infiltrate without glomerular lesions (Masson trichrome, ×100).


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**Table 2.** Cases showing overlap features between IgG4-TIN and TIN of autoimmune diseases

| | Age | Sex | Serum IgG (mg/dL) | Serum IgG4 (mg/dL) | Antibody | SCr (mg/dL) | Proteinuria | Systemic complications | IgG4/ HPF | IgG4/ IgG (%) | IF | EM | Diagnosis of kidney biopsy | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IgG4-RD and Sjogren's syndrome | 49 | F | 6,000 | 2,790 | Anti–SS-A (1:16) | - | - | Chronic hepatitis (3), portal hypertension (2), retroperitoneal ficrosis (1), renal involve (1) | 21.7 | 42.1 | - | - | - | Yamamoto *et al.*[47] |
| | 43 | F | 1,898 | 188 | Anti–SS-A (1:16) | - | - | | - | - | - | - | - | |
| | 48 | F | 3,009 | 768 | Anti–SS-A (1:4) | - | - | | 11.3 | 23.5 | - | - | - | |
| | 56 | F | 1,890 | 694 | Anti–SS-A (1:16) | - | - | | 9.7 | 19.4 | - | - | - | |
| | 59 | M | 1,880 | 339 | Anti–SS-A (1:64) | - | - | | - | - | - | - | - | |
| | 73 | M | 1,912 | 374 | Anti–SS-A (1:16) | - | - | | 6.7 | 21.3 | - | - | - | |
| | 61 | M | 2,558 | 774 | Anti–SS-A (1:16) | - | - | | 14.7 | 33.4 | - | - | - | |
| IgG4-RD and Sjogren's syndrome | 62 | F | 8,478 | 647 | ANA (1:10,240, homogeneous), anti–SS-A (+), anti–SS-B (+) | 0.92 | 0.82 g/gCr | General malaise, dry mouth, Raynaud's phenomenon, anemia, lower extremity weakness, hypergammaglobulinemia | 15 | - | No immunoglobulin or complement deposition | - | Chronic plasma cell-rich TIN | Kawano *et al.*[45] |
| IgG4-RD and Churg-Strauss Syndrome | 68 | F | 1,997 | 275 | ANCA (–), anti–SS-A (–), RF (+) | 0.9 | 1.2 g/day | Asthma, multifocal pulmonary infiltrates, marked eosinophilia, a rash on feet, right median nerve paralysis, salivary gland swelling | - | 10 | IgG, C3 (granular, capillary), IgG1, IgG4 (+) | Electron-dense to electron-lucent subepithelial deposits in glomerular capillary walls | MGN (stage III-IV) with eosinophil-rich TIN | Ayuzawa *et al.*[53] |
| IgG4-RD and Lupus nephritis | 71 | F | IgG1: 1,230, IgG2: 735, IgG3:418 | 37.1 | ANA (1.320 homogeneous) ANCA (–), anti–SS-A (–), anti–SS-B (–), anti-dsDNS (–), anti-Sm (–), anti-GBM (–) | 9.65 | 2.6 g/day | Abdominal pain, vomiting, diarrhea, epigastric tenderness, bilateral lower extremity pitting edema, marked leukocytosis, hypoalbuminemia, no skin changes | 13 | - | IgG, K, L (2+, granular, mesangial), IgM, IgA, C3 (1+, granular, mesangial) | Small paramesangial and scattered small electron dense to electron lucent subepithelial and intramembra-nous deposits | IgG4-related TIN with MGN, and/or lupus membranous nephritis with TIN | Zaarour *et al.*[54] |

IgG4-TIN, tubulointerstitial nephritis with dominant IgG4-positive cell infiltrate; SCr, serum creatinine; HPF, high-power field; IF, immunofluorescence; EM, electron microscopy; IgG4-RD, IgG4-related disease; ANA, antinuclear antibodies; TIN, tubulointerstitial nephritis; ANCA, anti-neutrophil cytoplasmic antibody; RF, rheumatoid factor; MGN, membranous nephropathy; GBM, glomerular basement membrane; K. kappa light chain; L, lambda light chain.

positive plasma cells was suggested as a form of IgG4-TIN,[55] but it was not supported by others.[56] Sakairi *et al*.[57] reported a case of ANCA-negative renal small-vessel vasculitis with IgG4-TIN. Recently, a case of multiple organ involvement remarkably similar to that of IgG4-RD was reported. The patient showed multiple hypodense renal lesions in radiographic examination and lymphoplasmacytic infiltrates with storiform fibrosis, but did not have accompanying elevated serum IgG4 and IgG4-positive plasma cell infiltration.[58] IgG4-TIN was reported in a patient with chronic lymphocytic leukemia,[59] and in a renal allograft patient.[60]

## CLINICAL AND HISTOLOGICAL OVERLAP BETWEEN IMMUNOGLOBULIN G4-TUBULOINTERSTITIAL NEPHRITIS AND TUBULOINTERSTITIAL NEPHRITIS OF AUTOIMMUNE DISEASES

Cases showing clinical, laboratory, and histological overlap of IgG4-TIN and TIN of autoimmune diseases have been introduced in previous sections,[24,45,47,53] and summarized in Table 2. These overlapping features may partly have roots in autoimmune mechanisms. IgG4-RD demonstrates immunologic derangement in cytokine profiles and activation of regulatory T cells.[2] Practically, the overlap causes dilemma and difficulties in diagnosis. Even though each disease accompanies unique histological features, confirmatory diagnosis is made by the diagnostic criteria of each disease. The ACR and SLICC criteria for SLE have been well established and used, but they have been modified by consensus among the experts, statistical results, and pathogenetic mechanisms.[61] Sjögren syndrome also has complex diagnostic criteria incorporating clinical features and histological findings. As mentioned previously, the diagnostic criteria of IgG4-RD are also composed of a combination of histological, clinical, imaging, and laboratory features. Furthermore, these complex diagnostic criteria may evolve as our understanding of the pathogenetic mechanisms and clinical course of the disease extends, as in SLE. In fact, some cases that had originally been

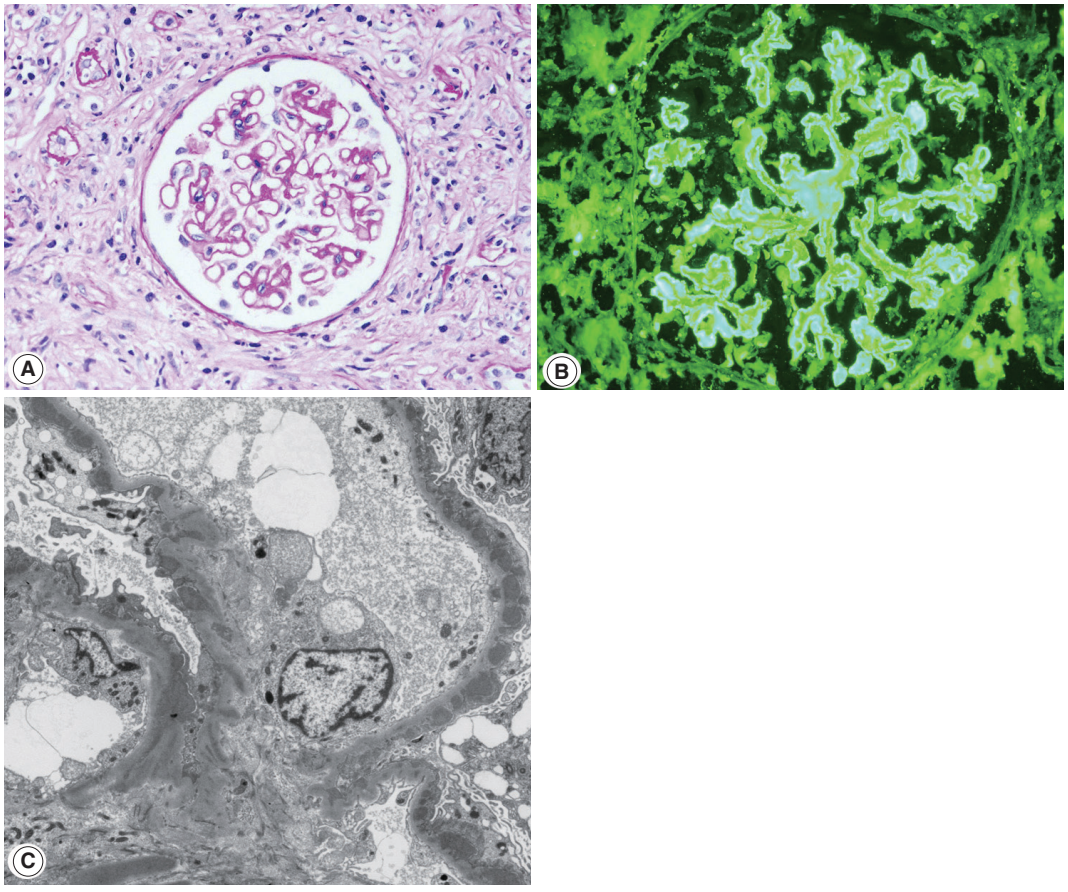


**Fig. 4.** Membranous nephropathy associated with IgG4-TIN. (A) Glomerular basement membrane is thickened with occasional spikes (periodic acid-Schiff, ×200). (B) Granular staining of IgG is present along the peripheral capillary walls (IgG, ×200). (C) Subepithelial electron-dense deposits are seen (×3,000).


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diagnosed as rheumatoid arthritis, Sjögren syndrome or antiphospholipid syndrome were re-categorized into IgG4-RD.[62] Even if we apply the above diagnostic criteria, symptoms are protean and laboratory abnormalities and organ involvement may vary in each individual patient. Clinical and laboratory findings supportive of autoimmune diseases or IgG4-RD may not be apparent at the time of biopsy.[45] Furthermore, in some cases it is not possible to distinguish IgG4-TIN from TIN of autoimmune diseases due to overlapping clinical and laboratory features. Cases in this gray zone should be collected and reserved in a separate category until we have more clear understanding on the pathogenetic mechanisms of IgG4-RD and so we can unveil the possible link between them.

## GLOMERULONEPHRITIS ASSOCIATED WITH IMMUNOGLOBULIN G4-TUBULOINTERSTITIAL NEPHRITIS

Glomerulonephritis may develop in the setting of IgG4-RD. Membranous nephropathy is the most common, and about 30 cases have been reported in the English literature so far.[26,63-69] Membranous nephropathy presented concurrently with TIN in most case reports, but in one collective series, four of nine cases presented with only glomerulonephritis.[15] Nephrotic syndrome is a frequent manifestation at the time of biopsy.[15] Glomerular histology is similar to that of idiopathic membranous nephropathy, except for a negative staining against phospholipase A2 receptor (PLA2R) antibodies. By light microscopy, glomerular cellularity is normal and the basement membrane is mildly thickened with occasional spikes (Fig. 4A). Immunofluorescence shows granular staining of IgG along the peripheral capillary walls (Fig. 4B). IgG4 is usually dominant among the IgG subclasses and C3 deposits are frequent. On electron microscopy, electron dense-deposits are present mainly in the subepithelial and occasionally in the intramembranous areas, ranging from stage I to III.

Membranous nephropathy of IgG4-RD is sometimes difficult to differentiate from lupus membranous nephritis. Although glomerular IgA, IgM, or C1q deposits are rare in IgG4-RD, strong C1q deposits have been reported in a few cases of IgG4-RD. Mesangial or subendothelial deposits were present in three and four of nine cases of membranous nephropathy associated with IgG4-RD, respectively.[15] Tubuloreticular inclusions, which are regarded as a characteristic feature of lupus nephritis,[70] were observed in one case of IgG4-RD.[15] Some authors proposed differential IgG subclass staining, which demonstrated strong IgG2 staining in lupus but not in IgG4-RD. However, it has not been verified in other studies.[15] IgG3 staining intensity similar to or exceeding that of IgG4[71] or IgG2[72] was reported in membranous lupus nephritis, but its comparison with that in IgG4-RD has not been done. Similar to TIN, distinction between membranous nephropathy associated with IgG4-RD and lupus membranous nephritis could not be made with certainty in a few cases. In a recent report of a case of membranous nephropathy, clinical features of both SLE and IgG4-RD were present.[69] Features suggestive of SLE were vitiligo, elevated ESR and CRP, hypocomplementemia, positive ANA and weakly positive anti-dsDNA antibody and atypical pANCA, while increased serum IgG4 levels, sialadenitis, lymphadenopathy, large kidneys, and marked hepatomegaly favored IgG4-RD. We experienced a case of membranous nephropathy in a patient satisfying ARA and SLICC criteria for SLE, but the patient also had retroperitoneal fibrosis.

Except for membranous nephropathy, Henoch-Schönlein purpura nephritis,[73-75] membranoproliferative,[76] and endocapillary proliferative glomerulonephritis[77] have been reported anecdotally. Cases with Henoch-Schönlein purpura nephritis had mesangial IgA deposition in addition to TIN. It is unclear whether these glomerulonephritides have a direct relation with IgG4-TIN or develop co-incidentally. In a case of endocapillary proliferative glomerulonephritis with crescent formation, the patient had hydroureteronephrosis due to retroperitoneal fibrosis and elevated circulating immune complexes,[77] which might raise a suspicion of urinary tract infection causing glomerulonephritis.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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■ ORIGINAL ARTICLE ■

# Review of Medical Advisory Services by the Korean Society of Pathologists from 2003 to 2014

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**Background:** Since 2003, the Korean Society of Pathologists (KSP) has been officially providing medical advisory services (MAS). We reviewed the cases submitted to the KSP between 2003 and 2014. **Methods:** In total, 1,950 cases were submitted, most by private health insurance companies. The main purposes of the consultations were to clarify the initial diagnoses and to assign a proper disease classification code. We comprehensively reviewed 1,803 consultation cases with detailed information. **Results:** In spite of some fluctuations, the number of submitted cases has been significantly increasing over the 12 study years. The colon and rectum (40.3%), urinary bladder (14.2%), and stomach (6.9%) were the three most common tissues of origin. The most common diagnoses for each of the three tissues of origin were neuroendocrine tumor (50.7%), non-invasive papillary urothelial carcinoma (70.7%), and adenocarcinoma (36.2%). Regardless of the tissue of origin, neuroendocrine tumor of the digestive system was the most common diagnosis (419 of 1,803). **Conclusions:** In the current study, we found that pathologic consultations associated with private health insurance accounted for a large proportion of the MAS. Coding of the biologic behavior of diseases was the main issue of the consultations. In spite of the effort of the KSP to set proper guidelines for coding and classification of tumors, this review revealed that problems still exist and will continue to be an important issue.

**Key Words:** Medical advisory service; Pathologic consultation; Private health insurance

Advisory services are widely used in the field of medicine in many countries. Doctors commonly use consultations, especially in surgical pathology practice. In the United States, the Association of Directors of Anatomic and Surgical Pathology has defined various kinds of pathology consultations and devised regulations to control the quality of each type.[1-3]

In Korea, pathologic consultations by doctors have also been performed in daily practice. However, most of these consultations have been conducted informally, without regulations or proper fees. Therefore, Korean pathologists agreed with the necessity for an official pathology advisory service system and organized the Medical Advisory Committee (MAC) as a subdivision of the Korean Society of Pathologists (KSP) in 2003. The MAC has been officially providing various kinds of advisory services, focusing on diagnostic pathology, since 2003. They have restrained the extent of users and contexts of pathologic consultations and have established detailed regulations. According to these regulations, certain individuals or institutions can use the consultation services: health and medical institutions run by government agencies such as the Ministry of Health and Welfare or community health centers; investigative authorities such as departments of prosecution or police departments; judicial offices; the Korean Medical Association; private insurance companies; specific institutions or individuals who are allowed to use the services of the KSP; and the members of the KSP.

The contents of consultations are defined by the third provision of the regulations of the medical advisory services (MAS): classification and evaluation of the grade of malignant or premalignant lesion; confirmation of a pathologic diagnosis, including review of slides or pathologic reports; pathologic review of controversial cases among pathologists or clinicians; consultation about rare diseases; and other medical questions allowed by the MAC.

The consultation process is quite simple (Fig. 1). After a client requests a pathologic consultation with the KSP, the advisory manager who is appointed by the MAC reviews the contents of the consultation. Then, he or she designates a specific pathologist as a consultant, considering his or her experience and subspecialty in pathologic fields. The appointed pathologist must have more than 10 years of experience as a surgical pathologist of the specific subspecialty. There must be no conflicts of interest between the consultant and client. After reviewing the consult-

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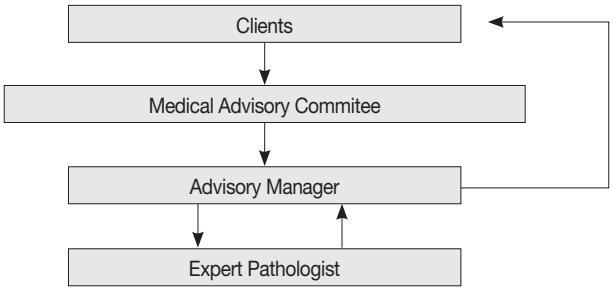


**Fig. 1.** Schematic flow chart of the medical advisory service system of the Korean Society of Pathology (KSP).

ed case, the assigned pathologist should report the result to the advisory manager within 2 weeks. Then, the advisory manager informs the clients of the result.

In this review, we discuss the medical advisory service system of Korea provided by the KSP and comprehensively review the consultation cases from 2003 to 2014.

## MATERIALS AND METHODS

The consultation cases are stored in a web-based medical advisory service system (http://jamoon.pathology.or.kr) that revealed 1,950 cases sent to the MAC from 2003 to 2014 (Fig. 2). We obtained the following specific information for each case: person who submitted the case, date on which the case was sent, assigned consulting pathologist, tissue of origin for the submitted case, and final diagnosis. After 46 cases with missing data were excluded from the review, 1,904 cases were reviewed and analyzed for this study. This study was approved by the Institutional Review Board of Konkuk University Hospital (KUH1210045).

Although various institutions and individuals were allowed to use the services, all but nine consultations were requested by private health insurance companies or insurance adjustment companies. The other nine cases were submitted by district courts or legal agencies.

The questions asked about the submitted cases were also limited. The major contents of the consultations were the same in most of the cases: "What is the precise pathologic diagnosis of the patient's illness?" and "Which classification code should be given considering the biologic nature and behavior of the illness?" To obtain answers to these questions, the submitters asked that the surgical pathology or cytopathology slides and original pathologic reports be reviewed, and that the consulting pathologist clarify the degree of malignancy using an accurate disease classification code based on the International Classification of Diseases for Oncology (ICD-O)[4] or the Korean Classification of Diseases (KCD).[5]

## RESULTS

### Number of consultation cases and distribution of tissues of origin

The number of submitted cases between 2003 and 2014 is presented in Fig. 2A. Although there was some fluctuation between 2007 and 2013, the number of submitted cases has been significantly increasing over the 12 study years, from four cases in 2003 to 296 cases in 2014.

We analyzed the tissues of origin of 1,904 cases from 2003 to 2014. The distributions of tissues of origin were slightly different throughout the years (Fig. 2A, Electronic Supplementary Table S1). The three most common tissues of origin from 2003 to 2014 were the colon and rectum (767, 40.3%), the urinary bladder (271, 14.2%), and the stomach (132, 6.9%). Cases with these three tissues of origin comprised more than half of the entire set of submitted cases. The next most common tissues of origin included the breast, ovary, thyroid, and uterus. Fig. 2B shows the organ distribution of the 1,904 cases submitted from 2003 to 2014.

### Tissue of origin-specific diagnoses

Among the 1,904 cases, the requests for 22 cases were not for a review of specific slides or pathologic reports. The contents of these consultations were questions about definition, biologic behaviors, or diagnostic methods for specific diseases. In 42 cases, submitters asked for a review of the cytopathologic diagnosis: two provided cervicovaginal smear slides, and 40 provided fine-needle aspiration slides of the thyroid. Thus, we excluded these 64 cases. In 37 of the remaining 1,840 cases, advisors could not provide a conclusive diagnosis because submitters did not provide appropriate information such as adequate pathology slides, slides with sufficient tissue, or complete clinical information. Finally, 1,803 cases were reviewed and classified based on the provided pathologic diagnosis. We analyzed these cases based on tissue of origin (Table 1, Electronic Supplementary Table S2–S21). For convenient presentation, we unified the diagnostic terms of neoplasms according to the most recently updated World Health Organization (WHO) classifications of tumors of each system.

#### *Colon and rectum*

The two main diagnoses in this category were "adenocarcinoma" and "neuroendocrine tumor (NET)," accounting for 42.5%


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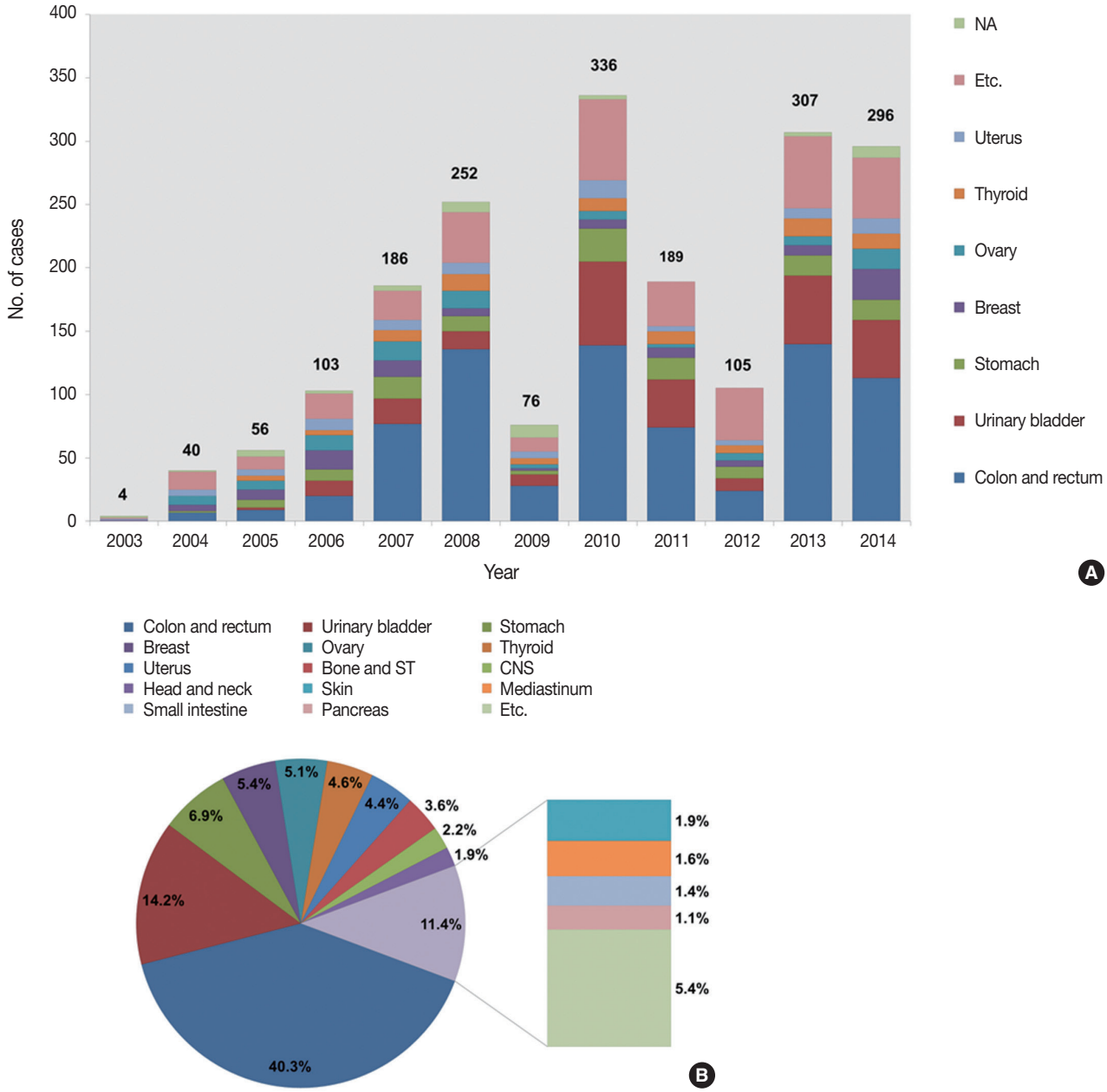


**Fig. 2.** Number of consultation cases from 2003 to 2014 and their tissues of origin. (A) The number of consultation cases has increased since 2003, although there are some variations. The colon and rectum have been the most common tissues of origin in recent years. (B) Of the entire set of consultation cases during the 12 study years, the colon and rectum were the most common tissues of origin, followed by the urinary bladder and stomach. NA, not applicable; ST, soft tissue; CNS, central nervous system.

(321 of 755) and 50.7% (383 of 755) of cases, respectively. Sixty-eight percent of the adenocarcinoma cases were "adenocarcinoma *in situ*" (263 of 321). The other 38.0% were early-stage tumors with only mucosal or submucosal invasion. In terms of NET, 78.6% originated from the rectum (302 of 383).

*Urinary bladder*

The urinary bladder was the second most common tissue of origin. Most of the cases in this category were urothelial carcinoma (from non-invasive papillary urothelial carcinoma to invasive urothelial carcinoma). The single most common entity was "non-invasive papillary urothelial carcinoma, low grade," and it accounted for 70.7% (186 of 263) of the urinary bladder cases. The second-most common (29 of 263, 11.0%) was "papillary urothelial neoplasm with low malignant potential."

**Table 1.** Diagnoses of consultation cases of the three major tissues of origin: the colon and rectum, urinary bladder, and stomach

| Tissue of origin-specific diagnoses | No. of cases |
|---|---|
| Colon and rectum | |
| Hyperplastic polyp | 4 |
| Tubular or tubulovillous adenoma | |
| Low-grade dysplasia | 11 |
| High-grade dysplasia | 28 |
| Adenocarcinoma | |
| Adenocarcinoma *in situ* | 263 |
| Adenocarcinoma with submucosal invasion | 58 |
| Neuroendocrine tumor | |
| Colon | 81 |
| Rectum | 302 |
| Gastrointestinal stromal tumor | |
| Low risk | 3 |
| Intermediate risk | 1 |
| Extranodal marginal zone lymphoma | 2 |
| Chronic inflammation | 1 |
| Total | 755 |
| Urinary bladder[a] | |
| Urothelial proliferative lesion | |
| Urothelial hyperplasia | 1 |
| Urothelial papilloma | 4 |
| Urothelial papilloma with atypia | 2 |
| Urothelial dysplasia | 1 |
| PUNLMP | 29 |
| Non-invasive papillary urothelial carcinoma, LG | 186 |
| Non-invasive papillary urothelial carcinoma, HG | 8 |
| Invasive urothelial carcinoma | 22 |
| Urothelial carcinoma *in situ* | 4 |
| Squamous cell carcinoma *in situ* | 1 |
| Metastatic adenocarcinoma | 1 |
| Cystitis | 4 |
| Total | 263 |
| Stomach | |
| Tubular adenocarcinoma | 30 |
| Signet ring cell carcinoma | 4 |
| Adenocarcinoma *in situ* | 12 |
| Tubular adenoma with high-grade dysplasia | 3 |
| Tubular adenoma with low-grade dysplasia | 2 |
| Gastrointestinal stromal tumor | |
| Very low risk | 8 |
| Low risk | 13 |
| Intermediate risk | 9 |
| High risk | 7 |
| Unknown | 3 |
| Extranodal marginal zone lymphoma of MALT | 10 |
| Neuroendocrine tumor | 15 |
| Granular cell tumor | 1 |
| Leiomyoma | 2 |
| Schwannoma | 1 |
| Nonneoplastic polyp[b] | 3 |
| Chronic gastritis | 3 |
| Total | 127 |

PUNLMP, papillary urothelial neoplasm with low malignant potential; LG, low grade; HG, high grade; MALT, mucosa-associated lymphoid tissue.
[a]All diagnoses are based on the 2004 World Health Organization classification; [b]Two fundic gland polyps and 1 hyperplastic polyp are included in this category.

*Stomach*

As a single entity, "adenocarcinoma" was the most common diagnosis of the cases in this category (46 of 127, 36.2%). Adenocarcinoma *in situ* was also a frequent diagnosis (12 of 46, 26.1%), followed by gastrointestinal stromal tumor (GIST) and NET (40 of 127, 31.5% and 15 of 127, 11.8%, respectively). We were able to obtain the risk classification of 37 submitted GISTs based on the National Institutes of Health (NIH) consensus criteria, including tumor size and mitotic activity.[6,7] Based on these criteria, tumors in the low risk group were the most common (13 of 40, 32.5%).

*Major diagnoses from other tissues of origin*

Electronic Supplementary Table S2–S21 details the specific diagnoses of the remaining tissues of origin. For breast as the tissue of origin, the diagnoses varied from benign ductal epithelial lesions such as columnar cell change to invasive ductal carcinoma (Electronic Supplementary Table S2). As a single entity, phyllodes tumor comprised the largest portion of the category (30 of 100, 30.0%). Among these, borderline phyllodes tumor (19 of 30, 63.3%) based on the WHO classification was the most common diagnosis.[8]

Female genital tissues, the ovary and uterus, were also common tissues of origin. In the ovary, surface epithelial neoplasm was the most common lesion (Electronic Supplementary Table S3). Forty of 95 cases (42.1%) were epithelial tumors, and half of these were borderline tumors. Granulosa cell tumor was also a common neoplasm (33 of 95, 34.7%), with the ovary as the tissue of origin. In cases with the uterus as the tissue of origin, those of the cervix were slightly more common than those of the corpus (43 vs 33). Among cases with the cervix as the tissue of origin, squamous cell carcinoma *in situ* (14 of 43) and microinvasive squamous cell carcinoma (12 of 43) together accounted for 60.5% of cases. Among cases with the corpus as the tissue of origin, those of smooth muscle neoplasm were the most frequent. In particular, cases of smooth muscle neoplasm of uncertain malignant potential were more commonly submitted than other entities in this category (Electronic Supplementary Table S5).

The thyroid was the fifth most common tissue of origin, followed by the ovary. While the main intent of the consultation in cases with other organs as the tissue of origin was confirmation of the original diagnosis or classification of the disease based on classification system, the content of consultation in cases of thyroid origin was questions regarding methods for diagnosing papillary carcinoma. One of the main questions in these cases was whether a pathologist could provide a definite diagnosis of


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papillary carcinoma with only a fine-needle aspiration cytology (FNAC) specimen. This question was included in the consultation of 34 cases. In 15 of these 34 cases, the consulting pathologists answered that experienced pathologists could confirm the diagnosis of papillary carcinoma without additional histologic confirmation. In the other 19 cases, the consultants replied that additional histologic confirmation was needed to confirm the diagnosis because of the possible discrepancies between the cytology reports and the surgical pathology reports.

In other categories, most of the users provided surgical pathology slides. However, in the thyroid category, 46.0% of the users (40 of 87) provided cytology slides of FNAC specimens. According to the Bethesda system for reporting thyroid cytopathology,[9] 31 of 40 cases belonged to category VI (malignant), and all of them were initially diagnosed as "papillary carcinoma." Nine of the 40 cases belonged to category V (suspicious for malignancy), and most of them were initially diagnosed as "suspicious for papillary carcinoma" (Electronic Supplementary Table S4).

Bone, soft tissue, and central nervous system were also common tissues of origin. In these categories, there was no predominant diagnosis, but a variety of rare tumors were submitted to confirm the original diagnosis (Electronic Supplementary Tables S6, S7). Most of the minor categories of tissues of origin such as head and neck, skin, kidney, and gallbladder included several minor diagnoses, without one predominant diagnosis. However, the mediastinum, small intestine, appendix, and pituitary gland had predominant diagnoses. Most of the cases with the mediastinum as the tissue of origin were thymomas (26 of 30, 86.7%), and "pituitary adenoma" was the only diagnosis in cases with the pituitary gland as the tissue of origin (5 of 5, 100%). In the small intestine category, GIST (13 of 24, 54.2%) and NET (9 of 24, 37.5%) comprised most of the diagnoses. All submitted cases with the appendix as the tissue of origin were either NET (7 of 10, 70.0%) or low-grade appendiceal mucinous neoplasm (3 of 10, 30.0%).

Across the entire dataset, regardless of tissue of origin, NET of the digestive system was the most common diagnosis. Four hundred nineteen of 1,803 cases (23.2%) were NETs from various organs. Adenocarcinoma of the digestive system was the next most common diagnosis (20.6%, 372 of 1,803). Non-invasive papillary urothelial carcinoma from the urinary system was also frequently diagnosed, representing the third most frequent diagnosis and accounting for 20.0% (198 of 1,803) of the entire set of cases.

## DISCUSSION

In Korea, most surgical pathology consultations are divided into two types. The first type is the so-called institutional pathology consultation or second opinion of pathologic slides.[10] This type of consultation is common in cases where a pathological diagnosis is made at one hospital, and subsequent therapy is provided at another. In such a situation, the pathologists at the new hospital are usually asked to review the original pathologic reports and slides to confirm the diagnosis. Another type of consultation is usually requested by pathologists who encounter difficult cases. In such cases, Korean pathologists consult with other pathologists working in teaching hospitals. This type of consultation is usually termed as an extra-departmental pathology consultation or a personal consultation.[11] The ultimate purpose of these consultations is to improve diagnostic accuracy and provide the best treatment to patients. In general, appropriate consultations are regarded as a helpful step for reducing errors in surgical pathology.[12]

This review of the 12-year MAS by the KSP revealed several important facts. First, we discovered the existence of another type of consultation for the accurate classification and coding of diseases for insurance reimbursement. Except for nine cases, all consultation cases in the current study were submitted by private health insurance companies or insurance adjustment companies who were hired by patients or insurance companies. The main purpose of these consultations seemed to be adjustment of insurance payments based on the new disease codes. Several studies have retrospectively reviewed pathologic consultations. However, those studies were examining institutional or personal pathologic consultations with a medical purpose.[10,11,13-15] Most prior studies have focused on the quality of consultations and diagnostic discrepancy between the primary pathologist and consulting pathologist.[10,11,14] Thus, this is the first study on pathologic consultation for the purpose of reimbursement of health insurance.

In Korea, all citizens have to acquire mandatory National Health Insurance. The public sector of National Health Insurance covers only part of the entire medical expenditure; therefore, most citizens purchase supplementary private health insurance. The National Statistical Office reported that more than 64% of individuals purchased private health insurance in 2010. As a result of this demand, the private health insurance market is growing rapidly.[16] Conflicts about health insurance payments are also increasing. Both public and private forms of coverage for medical expense reimbursement are based on the disease clas-

sification code assigned by clinicians based on the pathologic reports.[17] Therefore, coding of tumors is an important issue in establishing insurance reimbursement. Therefore, pathologic consultation for insurance reimbursement purposes is expected to increase.

Second, certain types of tumors were frequently consulted. Regardless of the tissue of origin, NET of the digestive system was the most common diagnosis (419 of 1,803). The issue of staging and classification of NET is as yet unsettled.[18] In addition, the staging and classification systems of NET are various and complex. Many doctors still use different guidelines in classifying NETs, which might result in confusion in the clinical setting and coding. Han *et al*.[17] used an internet-based survey and reported coding discrepancy among endoscopists when diagnosing NETs in the lower gastrointestinal tract. When given the same pathology report of a G1 NET of 1.5 cm size, with submucosal invasion, no lymphovascular invasion, a Ki-67 index less than 1%, and a clear resection margin, 29.2% of endoscopists classified the tumor as malignant (-/3), 61.5% classified it as having uncertain behavior (-/1), and 8.9% classified it as benign (-/0). Our study demonstrates that the disagreement of coding of tumors due to a lack of a unified and clear classification system could eventually affect insurance reimbursement and became a social issue.

Adenocarcinoma *in situ* of the colon and rectum and non-invasive urothelial carcinoma of the urinary bladder were two of the most common diagnoses in the consulted cases. In addition, the main request was determining the behavior codes of these tumors. In Korea, the KCD is the standard disease coding system and was established after the translation of the ICD by Statistics Korea to allow communication in a common language across clinical settings.[5] The ICD-O is a fundamental classification system for tumors, the coding of which constitutes a dual classification system for both topography (site) and morphology (histology, behavior, and grading of malignancy). The ICD-O was originally developed for cancer registration, but it is also used by healthcare providers for quality control and by researchers for clinical trial recruitment, among other purposes.[19] The revised ICD-O-3 added a last fifth digit that represents the biologic behavior of tumors (Table 2).

**Table 2.** Behavior codes of the International Classification of Diseases-Oncology-3 (ICD-O-3)

| ICD-O-3 code | Disease |
|---|---|
| /0 | Benign |
| /1 | Uncertain whether benign or malignant (borderline malignancy, low malignant potential, uncertain malignant potential) |
| /2 | Carcinoma *in situ* (intraepithelial, noninfiltrating, noninvasive) |
| /3 | Malignant, primary site |
| /6 | Malignant, metastatic or secondary site |
| /9 | Malignant, uncertain primary or secondary site |

Traditionally, the behavioral nature of tumors is classified as malignant or benign. The two most important characteristics of a malignant tumor are local invasion and distant metastasis. In actual practice, the diagnosis and classification of tumors are not that simple. Neoplasms with uncertain behavior (-/1) or carcinoma *in situ* (-/2) also exist. Some malignant tumors can evolve from a pre-invasive stage referred to as carcinoma *in situ*, which means that the cancer cells display the cytologic features of malignancy without invasion of the basement membrane.[20] Adenocarcinoma *in situ* and non-invasive urothelial carcinoma are such examples. The -/2 (*in situ*) tumors show more favorable behavior than the -/3 (malignant) tumors. Based on this finding, private health insurances usually only reimburse 10% to 20% of the amount of reimbursement to patients with -/3 (malignant) tumors. However, the underlying concepts of these tumors cannot be easily understood by patients because -/2 (*in situ*) tumors are also referred to as "cancer" in general. Thus, in this kind of situation, patients should perhaps seek medical or legal advice to ensure that they are reimbursed correctly.

The ICD-O is a useful system for the purposes of an internationally unified principal of disease coding. However, it is difficult to ensure that doctors assign an identical code to the same tumor. This seems to be due to numerous co-existing classification systems and synonyms for the same tumor, and doctors have their own classification preferences. Physicians also have their own viewpoints on the prognosis and biologic behavior of tumors based on experiences, and research, but these viewpoints could be changed. New forms of tumors and new opinions about tumor behavior are continuously being proposed. Because the ICD-O-3 was created for the statistical analysis of tumor prevalence and death rates, it cannot satisfy all the various viewpoints of doctors. Sometimes it makes doctors confused when they give a code to the disease and causes coding discrepancies.

These coding discrepancies between doctors might give rise to conflicts between patients and health insurance providers. Even with the same condition, patients can receive different payments from insurance providers depending upon the code chosen by the clinician or that of the pathologist reporting the diagnosis. Many patients and private health insurance providers recognize the possibility of discrepancies and therefore use advisory services such as the KSP or individual pathologists to obtain a more profitable diagnosis or classification code. We expect that this kind of situation will increase as the private health in-


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surance market expands.

Through this review of 12 years of MAS provided by the KSP, we have recognized that consultations associated with reimbursement of private health insurance account for a large proportion of pathologic advisory services, and that the coding of tumors is an important issue in Korean society. In particular, the complex coding systems and coding discrepancies among clinicians and/or pathologists are problems that need to be solved. The best solution is to establish a better tumor classification and coding system that is able to reflect the biologic nature of tumors and is easily understood by non-experts.

In order to accomplish this, pathologists must play a role, because the classification of tumors should be based on cytopathologic characteristics that best reflect the biologic nature of tumors. Korean pathologists have been actively working toward this goal. In collaboration with the National Cancer Center, the KSP has participated in the confirmation of diagnostic terms; standardization of diagnostic formats; and clarification and assessment of multiple primaries, primary sites, ICD-O codes, and education of pathologists.[21] Several study groups of the KSP have proposed behavior codes for several tumors with controversies regarding classification and coding.[21-23]

Thanks to these efforts, there were only a few discrepancies in coding of the same tumor among different subspecialty pathologists in this review (data not shown). However, a small proportion of coding discrepancies existed in certain tumors such as NET and granulosa cell tumor of the ovary. These cases were mostly diagnosed and consulted before the unified guidelines were proposed by the KSP. We therefore presume that the effort of the KSP in proposing and presenting a simplified and unified classification and coding system is having a positive effect. Nevertheless, this review of the MAS of the KSP reveals that there are still problems with the classification and coding systems for neoplasms, and that they will continue to be important issues. Therefore, we should persist in our efforts to focus attention on and further improve these areas.

## Electronic Supplementary Material

Supplementary materials are available at Journal of Pathology and Translational Medicine (http://jpatholtm.org).

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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**Supplementary Table S1.** Number of cases from each tissue origin from 2003 to 2014

| Tissue origin | Year | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | Total |
| Colon and rectum | 0 | 7 | 9 | 20 | 77 | 136 | 28 | 139 | 74 | 24 | 140 | 113 | 767 |
| Urinary bladder | 0 | 0 | 2 | 12 | 20 | 14 | 9 | 66 | 38 | 10 | 54 | 46 | 271 |
| Stomach | 0 | 1 | 6 | 9 | 17 | 12 | 3 | 26 | 17 | 9 | 16 | 16 | 132 |
| Breast | 1 | 5 | 8 | 15 | 13 | 6 | 2 | 7 | 8 | 5 | 8 | 24 | 102 |
| Ovary | 0 | 7 | 7 | 12 | 15 | 14 | 3 | 7 | 3 | 6 | 7 | 16 | 97 |
| Thyroid | 0 | 0 | 4 | 4 | 9 | 13 | 5 | 10 | 10 | 6 | 14 | 12 | 87 |
| Uterus | 1 | 5 | 5 | 9 | 8 | 9 | 5 | 14 | 4 | 4 | 8 | 12 | 84 |
| Bone and ST | 0 | 2 | 1 | 3 | 6 | 7 | 0 | 11 | 10 | 9 | 12 | 8 | 69 |
| CNS | 0 | 2 | 2 | 1 | 3 | 5 | 1 | 10 | 1 | 5 | 5 | 7 | 42 |
| Head and neck | 0 | 5 | 2 | 1 | 2 | 1 | 2 | 7 | 3 | 1 | 8 | 4 | 36 |
| Skin | 0 | 0 | 1 | 3 | 2 | 7 | 1 | 6 | 3 | 3 | 6 | 4 | 36 |
| Mediastinum | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 6 | 4 | 4 | 2 | 9 | 31 |
| Small intestine | 0 | 0 | 0 | 2 | 4 | 4 | 2 | 3 | 2 | 1 | 3 | 5 | 26 |
| Pancreas | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 2 | 2 | 4 | 5 | 0 | 21 |
| Etc. | 0 | 4 | 3 | 6 | 6 | 11 | 3 | 19 | 10 | 14 | 16 | 11 | 103 |
| Total | 3 | 39 | 51 | 101 | 182 | 244 | 66 | 333 | 189 | 105 | 304 | 287 | 1,904[a] |

ST, soft tissue; CNS, central nervous system.
[a]36 of the 1,950 entire consulted cases without information about tissue origin were excluded from this table.

**Supplementary Table S2.** Diagnoses of the consulted cases from breast

| Diagnosis | No. of cases |
|---|---|
| Benign lesions | |
| Columnar cell change | 3 |
| Usual ductal hyperplasia | 5 |
| Fibrocystic change | 3 |
| Atypical ductal lesion | |
| Atypical ductal hyperplasia | 19 |
| Atypical papilloma | 2 |
| Flat epithelial atypia | 2 |
| Ductal carcinoma *in situ* | 27 |
| Microinvasive carcinoma | 1 |
| Invasive ductal carcinoma | 7 |
| Phyllodes tumor | |
| Benign | 10 |
| Borderline | 19 |
| Malignant | 1 |
| Total | 100 |

**Supplementary Table S3.** Diagnoses of the consulted cases from ovary

| Diagnosis | No. of cases |
|---|---|
| Epithelial neoplasm | |
| Brenner tumor | 1 |
| Serous borderline tumor | 16 |
| Mucinous carcinoma | 2 |
| Mucinous borderline tumor (including carcinoma *in situ*) | 24 |
| Mucinous borderline tumor with microinvasion | 2 |
| Mucinous cystadenoma | 1 |
| Seromucinous borderline tumor | 1 |
| Clear cell carcinoma | 1 |
| Clear cell borderline tumor | 1 |
| Endometrioid carcinoma | 1 |
| Sex cord stromal tumor | |
| Fibrothecoma | 1 |
| Fibroma | 1 |
| Granulosa cell tumor | 33 |
| Sertoli-Leydig cell tumor | 3 |
| Steroid cell tumor | 1 |
| Germ cell tumor | |
| Mature teratoma | 1 |
| Somatic type tumor arising from mature teratoma | |
| Carcinoid tumor | 2 |
| Papillary carcinoma | 1 |
| Squamous cell carcinoma | 1 |
| Xanthogranulomatous inflammation | 1 |
| Total | 95 |

**Supplementary Table S4.** Diagnoses of the consulted cases from thyroid

| Diagnosis | No. of cases |
|---|---|
| Adenomatous goiter | 4 |
| Hyalizing trabecular tumor | 2 |
| Follicular adenoma | 5 |
| Hurthle cell adenoma | 1 |
| Follicular carcinoma | 1 |
| Papillary carcinoma | 21 |
| Well differentiated tumor with UB | 2 |
| Total | 36[a] |

UB, uncertain behavior.
[a]Forty cases for the review of fine-needle aspiration slides were excluded.

**Supplementary Table S5.** Diagnoses of the consulted cases from uterus

| Diagnosis | No. of cases |
|---|---|
| Cervix | |
| Squamous cell lesions | |
| CIN[a] | 7 |
| Squamous cell carcinoma *in situ* | 14 |
| Microinvasive squamous cell carcinoma | 12 |
| Invasive squamous cell carcinoma | 2 |
| Glandular lesions | |
| Endocervical glandular hyperplasia | 1 |
| Adenocarcinoma *in situ* | 1 |
| Microinvasive adenocarcinoma | 4 |
| Invasive adenocarcinoma | 2 |
| Corpus | |
| Endometrial lesions | |
| Endometrial hyperplasia with atypia | 4 |
| Endometrial adenocarcinoma | 4 |
| Adenofibroma with atypia | 1 |
| Endometrial stromal nodule | 1 |
| Endometrial stromal sarcoma | 1 |
| Smooth muscle neoplasm | |
| Cellular leiomyoma | 2 |
| Epithelioid leiomyoma | 1 |
| Mitotically active leiomyoma | 2 |
| STUMP | 9 |
| Uterine tumor resembling ovarian sex cord tumor | 1 |
| Trophoblastic lesions | |
| H-mole, invasive | |
| Placental site trophoblastic tumor | 4 |
| Epithelioid trophoblastic tumor | 1 |
| Total | 76[b] |

CIN, cervial intraepithelial neoplasia; STUMP, smooth muscle tumor of uncertain malignant potential; H-mole, hydatidiform-mole.
[a]CIN I was regarded as benign lesion and CIN category was consisted of 1 CIN I and 6 CIN III; [b]Two cases for the review of cervicovaginal cytology slides were excluded.

**Supplementary Table S6.** Diagnoses of the consulted cases from bone and soft tissue

| Diagnosis | No. of cases |
|---|---|
| Bone | |
| Chondroid lesion | |
| Osteochondroma | 2 |
| Enchondroma | 4 |
| Epithelioid angiosarcoma | 1 |
| Langerhans cell histiocytosis | 1 |
| PEComa | 1 |
| Ewing sarcoma/PNET | 1 |
| Giant cell tumor (osteoclastoma) | 1 |
| Simple bone cyst | 1 |
| Fibrous dysplasia | 1 |
| Bone infarct | 1 |
| Metastatic tumor | 2 |
| Soft tissue | |
| Atypical lipomatous tumor/WD liposarcoma | 5 |
| Fibromatosis | 5 |
| Extrapleural solitary fibrous tumor | 3 |
| Giant cell tumor of soft tissue | 1 |
| Myofibroma | 1 |
| STUMP | 2 |
| Rhabdomyosarcoma, spindle cell type | 1 |
| Kaposiform hemangioendothelioma | 1 |
| Epithelioid hemangioendothelioma | 2 |
| Schwannoma | 5 |
| Granular cell tumor | 1 |
| Malignant granular cell tumor | 1 |
| MPNST | 1 |
| Deep ("aggressive") angiomyxoma | 2 |
| Alveolar soft part sarcoma | 1 |
| Synovial sarcoma | 1 |
| Angiomatoid fibromyxoid tumor | 1 |
| Tenosynivial giant cell tumor | |
| Localized type | 6 |
| Diffuse type | 3 |
| Plexiform fibrohistocytic tumor | 1 |
| Benign reactive change | 3 |
| Total | 63 |

PNET, primitive neuroectodermal tumor; WD, well differentiated; STUMP, smooth muscle tumor with uncertain malignant potential; MPNST, malignant peripheral nerve sheath tumor.

**Supplementary Table S7.** Diagnoses of the consulted cases from central nervous system

| Diagnosis | No. of cases |
|---|---|
| Brain | |
| Glial tumor | |
| Pilocytic astrocytoma | 7 |
| Diffue astrocytoma | 3 |
| Menigioma | |
| Meningothelial | 3 |
| Transitional | 1 |
| Atypical | 2 |
| Chordoid | 1 |
| Rhabdoid | 1 |
| Craniopharyngioma | 1 |
| Choroid plexus papilloma | 1 |
| Pineal gland malignant tumor | 1 |
| Hemangioblastoma | 1 |
| Dermoid cyst | 1 |
| Immature teratoma | 1 |
| Germinoma | 1 |
| Malignant lymphoma | 2 |
| Metastatic carcinoma | 1 |
| Spinal cord | |
| Schwannoma | 5 |
| Neurofibroma | 1 |
| Diffue astrocytoma | 1 |
| Atypical meningioma | 1 |
| Mature teratoma | 1 |
| Arachnoid cyst | 1 |
| Hematoma | 1 |
| Total | 39 |

**Supplementary Table S8.** Diagnoses of the consulted cases from head and neck

| Diagnosis | No. of cases |
|---|---|
| Ocular lesions | |
| Extranodal marginal zone lymphoma | 2 |
| Invasive squamous cell carcinoma | 2 |
| Chronic dacryoadenitis | 2 |
| Nasal/paranasal cavity | |
| Squamous cell carcinoma | 2 |
| Spindle cell sarcoma | 1 |
| Diffuse large B cell lymphoma | 1 |
| Oral cavity | |
| Invasive squamous cell carcinoma | 2 |
| Squamous cell carcinoma *in situ* | 1 |
| Squamous papilloma | 1 |
| Cavernous hemangioma | 1 |
| Tuberculosis of palatine tonsil | 1 |
| Salivary gland | |
| Myoepithelioma | 2 |
| Carcinoma ex pleomorphic adenoma | 3 |
| Acinic cell carcinoma | 1 |
| Basal cell adenocarcinoma | 1 |
| Ear | |
| Neuroendocrine tumor | 1 |
| Squamous cell carcinoma | 1 |
| Adenoid cystic carcinoma | 1 |
| Larynx | |
| High grade dysplasia | 2 |
| Squamous cell carcinoma *in situ* | 4 |
| Invasive squamous cell carcinoma | 3 |
| Pharynx | |
| Mucoepidermoid carcinoma | 1 |
| Total | 36 |

**Supplementary Table S9.** Diagnoses of the consulted cases from skin

| Diagnosis | No. of cases |
|---|---|
| Keratinocytic lesions | |
| Keratoacanthoma | 1 |
| Basal cell carcinoma | 4 |
| Squamous cell carcinoma | 2 |
| Melanocytic lesions | |
| Atypical Spitz nevus | 1 |
| Dysplastic nevus | 1 |
| Melanoma *in situ* | 1 |
| Malignant melanoma | 1 |
| Appendigeal tumor | |
| Extramammary paget disease | 1 |
| Sebaceous carcinoma | 1 |
| Plemorphic adenoma (mixed tumor) | 1 |
| Hematolymphoid tumors | |
| Mycosis fungoides | 3 |
| Lymphomatoid papulosis | 1 |
| Soft tissue tumors | |
| Dermatofibroma (fibrous histocytoma) | 7 |
| DFSP | 5 |
| Tufted angioma | 1 |
| MPNST arising from neurofibromatosis | 1 |
| Granular cell tumor | 1 |
| Abcess | 1 |
| Epidermal cyst | 1 |
| Seborrehic keratosis | 1 |
| Total | 36 |

DFSP, dermatofibrosarcoma protuberance; MPNST, malignant peripheral nerve sheath tumor.

**Supplementary Table S10.** Diagnoses of the consulted cases from mediastinum

| Diagnosis | No. of cases |
|---|---|
| Thymoma | |
| Type AB | 8 |
| Type B1 | 7 |
| Type B2 | 7 |
| Type B3 | 3 |
| Type unknown | 1 |
| Schwannoma | 2 |
| Mature teratoma | 1 |
| Metastatic giant cell tumor of bone | 1 |
| Total | 30 |

**Supplementary Table S11.** Diagnoses of the consulted cases from small intestine

| Diagnosis | No. of cases |
|---|---|
| Gastrointestinal stromal tumor | |
| Very low risk | 1 |
| Low risk | 10 |
| Intermediate risk | 2 |
| Neuroendocrine tumor | 9 |
| Tubular adenoma with high grade dysplasia | 1 |
| Hamartoma | 1 |
| Total | 24 |

**Supplementary Table S12.** Diagnoses of the consulted cases from pancreas

| Diagnosis | No. of cases |
|---|---|
| IPMN | |
| Low-grade dysplasia | 3 |
| Intermediate-grade dysplasia | 5 |
| High-grade dysplasia | 1 |
| Mucinous cystic neoplasm | |
| Low-grade dysplasia | 1 |
| Intermediate-grade dysplasia | 1 |
| High-grade dysplasia | 1 |
| Neuroendocrine tumor | 5 |
| Solid pseudopapillary neoplasm | 4 |
| Total | 21 |

IPMN, intraductal papillary mucinous neoplasm.

**Supplementary Table S13.** Diagnoses of the consulted cases from lung and pleura

| Diagnosis | No. of cases |
|---|---|
| Lung | |
| Adnoecarcinoma *in situ*[a] | 7 |
| Invasive adenocarcinoma | 2 |
| Squamous cell carcinoma *in situ* | 1 |
| Extramedullary plasmacytoma | 1 |
| Langerhans cell histocytosis | 1 |
| Sclerosing pneumocytoma | 1 |
| Pulmonary hamartoma | 1 |
| Bronchiectasis | 1 |
| Pleura | |
| Well differentiated papillary mesothelioma | 1 |
| Solitary fibrous tumor | 2 |
| Total | 18 |

[a]Five of seven cases were diagnosed as non-mucinous bronchioloalveolar carcinoma before 2014.

**Supplementary Table S14.** Diagnoses of the consulted cases from kidney

| Diagnosis | No. of cases |
|---|---|
| Cystic, partially differenciated nephroblastoma | 2 |
| Chromophobe renal cell carcinoma | 1 |
| Papillary renal cell carcinoma | 1 |
| Non-invasive papillary urothelial carcinoma, LG | 1 |
| Malignant hemangioendothelioma | 1 |
| Epithelioid angiomyolipoma | 1 |
| Mixed epithelial and stromal tumor | 1 |
| Oncocytoma | 1 |
| Chronic pyelonephritis | 1 |
| Total | 10 |

LG, low grade.

**Supplementary Table S15.** Diagnoses of the consulted cases from appendix

| Diagnosis | No. of cases |
|---|---|
| Neuroendocrine tumor | 7 |
| Low grade appendiceal mucinous neoplasm | 3 |
| Total | 10 |

**Supplementary Table S16.** Diagnoses of the consulted cases from gallbladder

| Diagnosis | No. of cases |
|---|---|
| Adenoma | 3 |
| Biliary intraepithelial neoplasia, grade 3 | 1 |
| Adenocarcinoma in situ arising in adenoma | 2 |
| Cholecystitis | 3 |
| Adenomyomatous hyperplasia | 1 |
| Total | 10 |

**Supplementary Table S17.** Diagnoses of the consulted cases from lymph node

| Diagnosis | No. of cases |
|---|---|
| Maligant lymphoma | |
| Nodal marginal zone lymphoma | 3 |
| Classic Hodgkin lympoma | 2 |
| Langerhans cell histocytosis | 2 |
| Kimura disease | 1 |
| Dermatopathy lymphadenopathy | 1 |
| Total | 9 |

**Supplementary Table S18.** Diagnoses of the consulted cases from adrenal gland

| Diagnosis | No. of cases |
|---|---|
| Adrenal cortical adenoma | 1 |
| Oncocytic adrenal cortical tumor | 2 |
| Adrenal cortical carcinoma | 1 |
| Pheochromocytoma | 2 |
| Composite pheochromocytoma | 1 |
| Adenal cyst | 1 |
| Total | 8 |

**Supplementary Table S19.** Diagnoses of the consulted cases from liver

| Diagnosis | No. of cases |
|---|---|
| Dysplatic nodule, HG | 1 |
| Hepatocellular carcinoma | 1 |
| Biliary intraepithelial neoplasia, grade 1 | 1 |
| IPN with LG dysplasia | 1 |
| IPN with an associated invasive carcinoma | 1 |
| Epithelioid hemangioendothelioma | 1 |
| Total | 6 |

HG, high grade; IPN, intraductal papillary neoplasm; LG, low grade.

**Supplementary Table S20.** Diagnoses of the consulted cases from testis

| Diagnosis | No. of cases |
|---|---|
| Mature teratoma | 1 |
| Leydig cell tumor | 2 |
| Mucinous borderline tumor | 1 |
| Leiomyoma | 1 |
| STUMP | 1 |
| Total | 6 |

STUMP, smooth muscle neoplasm of uncertain malignant potential.

**Supplementary Table S21.** Diagnoses of the consulted cases from other tissue origins

| Diagnosis | No. of cases |
|---|---|
| Pituitary gland | |
| Pituitary adenoma | 5 |
| Ureter | |
| Non-invasive papillary urothelial carcinoma, LG | 3 |
| Invasive urothelial carcinoma | 1 |
| Bone marrow | |
| Myelodysplastic syndrome | 2 |
| Hairy cell leukemia | 1 |
| Hemophagocytic syndrome | 1 |
| Esophagus | |
| High grade dysplasia | 1 |
| Squamous cell carcinoma *in situ* | 1 |
| Leiomyoma | 1 |
| Peritoneum | |
| Pseudomyxoma peritonei | 2 |
| Malignant mesothelioma | 1 |
| Anus | |
| Squamous cell carcinoma *in situ* | 1 |
| Extrahepatic bile duct | |
| Adenocarcinoma | 1 |
| Vagina | |
| Reactive fibrotic tissue with necrosis | 1 |
| Prostate | |
| Prostatic glands and stroma with no tumor | 1 |
| Heart | |
| Myxoma | 1 |
| Parathyroid | |
| Parathyroid carcinoma | 1 |
| Total | 25 |

LG, low grade.

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■ ORIGINAL ARTICLE ■

# Eosinophils in Colorectal Neoplasms Associated with Expression of CCL11 and CCL24

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**Background:** A decrease in the number of tissue eosinophils is known to reflect the malignancy potential of neoplastic lesions and even prognosis. Increased levels of the chemokines CCL11 and CCL24 in serum and tissue are also known to have diagnostic value as serum tumor markers or prognostic factors. The aim of this study was to evaluate the correlation between the degree of tissue eosinophilia and the expression of these chemokines in the glandular and stromal cells of colorectal neoplastic lesions ranging from benign to malignant tumors. **Methods:** We counted the number of infiltrating eosinophils in neoplastic lesion tissue and we evaluated the expression of CCL11 and CCL24 in glandular cells and stromal cells by immunohistochemical staining. **Results:** The results showed that the number of eosinophils decreased significantly and the expression of CCL11 and CCL24 in glandular cells decreased with tumor progression, whereas the stromal expression of CCL11 and CCL24 appeared to increase. **Conclusions:** The discrepancy in CCL11 and CCL24 expression between glandular cells and stromal cells might shed light on how colorectal cancer evades the immune system, which would enable further development of immunotherapies that target these chemokines. Further research on eosinophil biology and the expression pattern of chemokines in tumor cells is needed.

**Key Words:** Eosinophils; CCL11; CCL24; Colorectal neoplasms

Eosinophils are bone-marrow–derived granulocytes present in peripheral blood and tissue that are primarily involved as effector cells in various conditions, including inflammatory diseases, allergic diseases, and parasitic infections.[1,2] Researchers have long thought that the main role of eosinophils was to act as effector cells in allergic reactions and helminth infections, but their role as antitumor effectors has recently been revealed.[3-6] Eosinophils function as antitumor effector cells in neoplastic lesions through a variety of eosinophil-derived mediators, including tumor necrosis factor alpha and granzyme-A.[3,7]

Changes in the number of tissue eosinophils have been reported in several types of cancer and premalignant lesions from a wide range of organs, including the head and neck and the gastrointestinal tract.[5,8-12] Some studies have shown that the degree of tissue eosinophilia correlates with cancer prognosis because of its significant association with response to chemoradiation therapy, operability, and even lymph node metastasis.[5,11,13-16] These findings suggest that the degree of eosinophilia could be used as a prognostic marker. Among the studies on eosinophils and neoplastic conditions, both increasing and decreasing trends in the number of eosinophils in colorectal neoplasms have been reported with regards to tumor progression. A histopathological study on tissue eosinophilia in colorectal neoplasms has revealed that the degree of eosinophilia differs according to the malignant potential of the lesion; the number of tissue-infiltrating eosinophils increases in low-grade dysplasia, decreases in high-grade lesions, and decreases even more in cancer cases, showing significant and rapid change compared to the surrounding normal tissues.[17-19] As mentioned earlier, more prominent eosinophilia in colorectal cancer is associated with a better prognosis, includ-

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ing significant improvement in the 5-year survival rate.

As the association between the infiltration of eosinophils and neoplasms is better understood, interest in how chemokines affect the differentiation and migration of eosinophils is increasing. Many kinds of chemokines are involved in the production, differentiation, and migration of eosinophils. Among the interleukins, interleukin (IL)-3 and IL-5 play major roles in the production and differentiation of eosinophils, and IL-4, IL-6, IL-11, and IL-12 are involved in their differentiation. A variety of C-C chemokines (named after the cysteine terminus residue near the C-terminus in the amino acid sequence), including C-C chemokine ligands-5 (CCL5, RANTES), CCL11 (eotaxin-1), CCL24 (eotaxin-2), and CCL26 (eotaxin-3), are involved in the differentiation and, in particular, the migration of eosinophils from the bone marrow to tissue stroma through enhancement and regulation of tethering, rolling, and endothelial adhesion of the eosinophils.[1,2]

Expression of CCL11 and CCL24 in the human gastrointestinal tract is higher than in other tissues in the normal state, and increases during inflammatory disease, allergic reaction, and helminth infections, in which eosinophils function as effector cells.[1,20-22] The concentration of CCL24 is shown to be elevated in tumor tissue, and is also elevated in the stromal cells of colorectal tumors.[23] As mentioned earlier, a decrease in tissue eosinophils was observed in colorectal adenocarcinoma, but tissue levels of CCL11 were found to be elevated. Therefore, there is a discrepancy between decreased tissue eosinophilia and the tissue levels of chemokines such as CCL11 and CCL24, which enhance the recruitment of eosinophils. Serum chemokine levels appear to be elevated in prostate and colorectal adenocarcinoma, and they have been proposed as serum tumor markers.[24-28]

In this study, we aimed to (1) evaluate the number of tissue-infiltrating eosinophils in colorectal tubular adenoma with low-grade dysplasia, tubular adenoma with high-grade dysplasia, and adenocarcinoma, and (2) evaluate the expression of CCL11 and CCL24 in colorectal neoplastic lesions by immunohistochemical staining to determine the correlation between the expression of cytokines, eosinophilia, and progression of neoplastic lesions. Even though much is known about the correlation between the prognosis and progression of colorectal neoplasms and eosinophilia, little is known about local expression of the chemokines associated with eosinophils. This study may provide insight into the relationship between tumor eosinophilia and local expression of chemokines, and thus explain the observed discrepancy between chemokine levels and tissue eosinophilia.

## MATERIALS AND METHODS

### Patients and tissue samples

Among the patients who underwent colonoscopic biopsy at Kyung Hee Medical Center and were diagnosed with colorectal tubular adenoma with any degree of dysplasia or colorectal cancer, a list of 50 patients was generated and their clinicopathological data were retrospectively collected. Patients were categorized into separate groups based on whether they had tubular adenoma with low-grade dysplasia, tubular adenoma with high-grade dysplasia, or adenocarcinoma. For adenocarcinoma cases, patients who had undergone resection were selected. Specimens with severe squeezing or cautery artifacts and specimens from patients with a history of other malignancies were excluded. For colorectal cancers, selection was performed among those who had undergone surgical resection and whose surgical specimens were retrieved from Kyung Hee Medical Center. Every diagnosis was reviewed and confirmed by pathologists. Each paraffin block from the specimen was cut into 4–5-μm thick sections and stained with hematoxylin and eosin for direct counting of eosinophils under microscopy. This study was approved by the Institutional Review Board (IRB) of Kyung Hee University (IRB 2015-08-039).

### Evaluation of tissue eosinophilia

Tissue eosinophils were counted directly on hematoxylin and eosin-stained slides of each specimen using an Olympus BX-53 microscope (Olympus, Tokyo, Japan). Eosinophils in the mucosa and submucosa were counted in three "hotspots" near the neoplastic lesion under a high-power field (×400). Controversial results were resolved by consensus of more than two pathologists using a multiview microscope.

### Immunohistochemical staining

Each specimen was prepared into 4–5-μm thick paraffin-embedded sections for immunohistochemical staining. Monoclonal mouse antibodies against CCL11 (LS-C139009, LifeSpan Biosciences, Seattle, WA, USA) and monoclonal mouse antibodies against CCL24 (LS-C104346, LifeSpan Biosciences) were used for immunohistochemical staining of the specimens. Unstained slides from each specimen were processed for 20 minutes in a pressure cooker for antigen retrieval. Immunohistochemical staining of all slides was performed using BOND-MAX (Leica Biosystems, Nusslock, Germany) with a dilution ratio of 1:200 for CCL11 and 1:800 for CCL24. Staining of CCL11 and CCL24 in glandular cells of neoplastic lesions was


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evaluated according to the number of glandular cells showing positive staining and the intensity of the staining over the slide. The number of positively stained glandular cells ranged between 1 and 60, and individual counts were scored as 0 (0–15 cells), 1 (16–30 cells), 2 (31–45 cells), or 3 (≥46 cells). Staining intensity was scored as 1 (faint), 2 (intermediate-strong), and 3 (strong granular). The sum of the immunohistochemical stain scores ranged from 1 to 6, and each summed score was then divided into categories of low (score 1–2), intermediate (score 3–4), and high (score 5–6). We also conducted immunohistochemical staining of CCL11 and CCL24 in stromal cells. Each specimen contained a different amount of stroma because most were endoscopically biopsied samples. Thus, expression in the stromal cells needed to be measured as a proportion rather than an absolute count. Positively stained stromal cells were counted under a microscope and scored as low, intermediate, or high according to the cutoff values of 5%, 15%, and > 30% for the percentage of cells showing positive staining relative to the total number of stromal cells in the high-power field.

### Statistical analyses

SPSS ver. 20.0 (IBM Co., Armonk, NY, USA) was used for the statistical analyses. Correlation between the number of eosinophils and tumor progression was analyzed with a one-way ANOVA. Correlation between the expression of CCL11 and CCL24 in glandular and stromal cells according to each different tumor progression group was analyzed via chi-square tests.

## RESULTS

Tissue eosinophilia in the neoplastic lesions was strikingly different according to progression of the lesion. Tubular adenoma with low-grade dysplasia included a stunning number of infiltrating eosinophils, whereas less eosinophils were present in cases of adenoma with high-grade dysplasia, and only a few eosinophils were counted in adenocarcinoma cases. These infiltrating eosinophils were mostly found adjacent to neoplastic lesions, close to neoplastic glands in tumor stroma.

One-way ANOVA analysis was performed to evaluate the differences, and the results confirmed that the number of eosinophils differed significantly according to the malignant potential of the lesion ($p < .001$) (Fig. 1).

Immunohistochemical staining of CCL11 in the glandular cells of neoplastic lesions appeared was strong and granular in cases of low-grade dysplasia (Fig. 2A). A mix of strong and faint staining was observed in high-grade dysplasia cases (Fig. 2B).

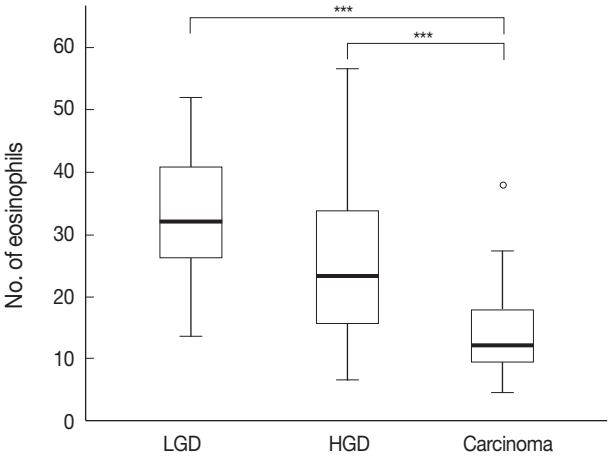


**Fig. 1.** The number of eosinophils in the colorectal neoplastic lesions: the number of infiltrating eosinophils increased significantly with the progression of colorectal neoplastic lesions. Asterisk (***) indicates $p < .001$ in each group. LGD, low grade dysplasia; HGD, high grade dysplasia.

In contrast, most of the staining in adenocarcinoma cases was weak and faint (Fig. 2C).

Expression of CCL24 in glandular cells of neoplastic lesions was similar to CCL11 expression. CCL24 staining revealed a mostly strong and granular pattern of expression in low-grade dysplasia cases (Fig. 2D), a mix of strong and weak staining in high-grade dysplasia cases, and an intermediate pattern in cases that are between low-grade dysplasia and adenocarcinoma (Fig. 2E). In contrast, a weak and faint pattern of staining was dominant in adenocarcinoma cases (Fig. 2F). Analysis of immunohistochemical staining showed significant differences between the groups ($p < .001$ in CCL11 and $p < .001$ in CCL24) (Table 1).

CCL24 staining in the stromal cells of patients with low-grade dysplasia was scarce and weak (Fig. 1G). Slightly more staining was observed in high-grade dysplasia cases (Fig. 2H), whereas stromal cells with CCL11 reactivity were frequently seen in adenocarcinoma cases (Fig. 2I). CCL11 expression appeared to increase with increased progression of the neoplastic lesion ($p < .001$) (Table 1).

CCL24 expression in stromal cells had a similar pattern to that of CCL11. CCL24-expressing stromal cells were scant and faintly stained in low-grade dysplasia cases (Fig. 2J), whereas a small number of positive stromal cells were observed in high-grade dysplasia cases (Fig. 2K). CCL24-positive stromal cells were frequently identified in adenocarcinoma cases (Fig. 2L). Stromal-CCL24 expression also increased significantly with tumor progression ($p < .001$) (Table 1).

Comparisons of tumoral and stromal expression of CCL11

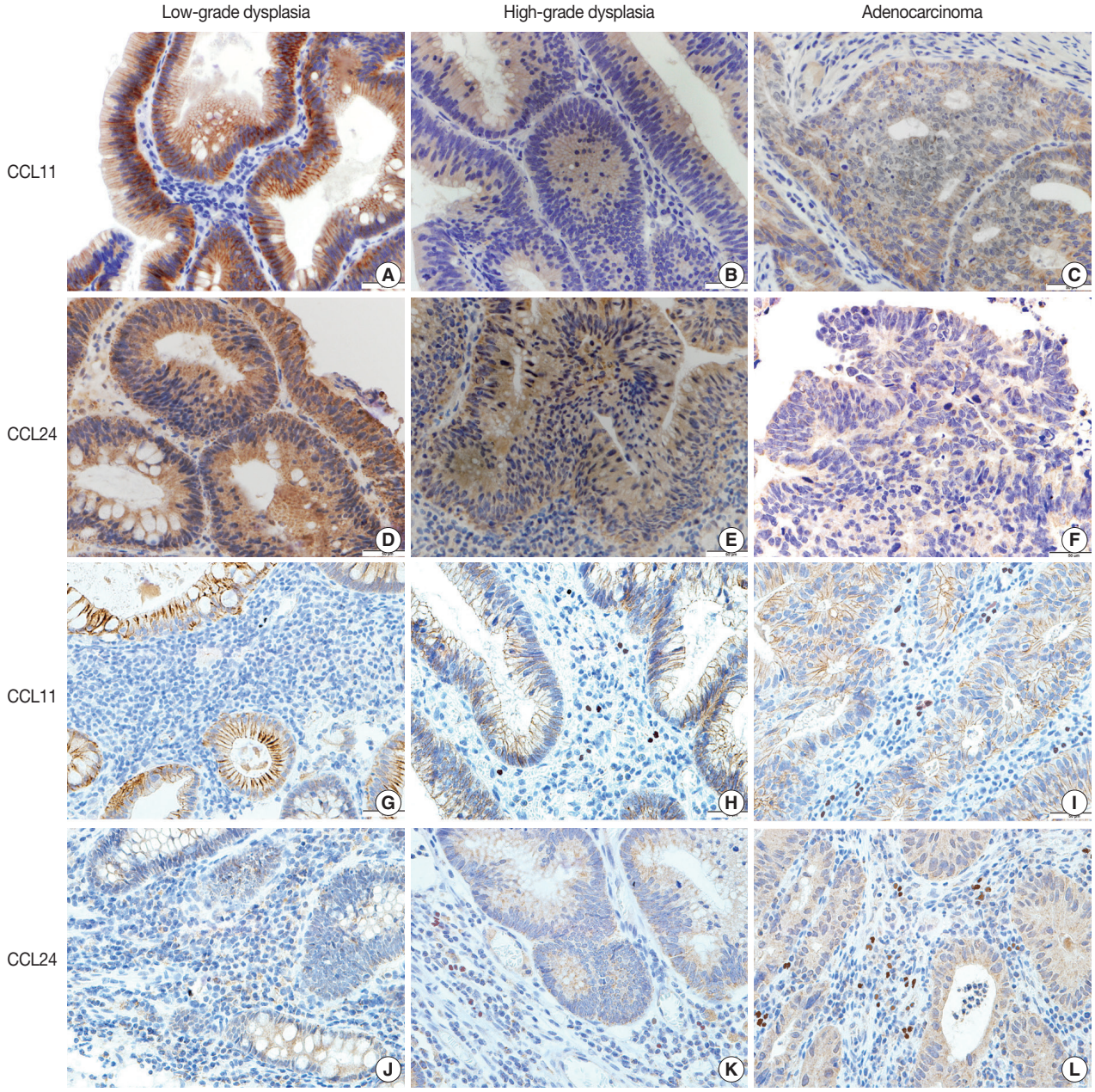


**Fig. 2.** Immunohistochemical staining of CCL11 and CCL24: immunohistochemical stains of CCL11 (A) and CCL24 (D) showed, in order of the strength of the observation, positivity in the tumor cells of colorectal tubular adenomas with low-grade dysplasia, adenomas with high-grade dysplasia (B, CCL11; E, CCL24), and adenocarcinoma (C, CCL11) and CCL24 (F). Immunohistochemical stains of CCL11 and CCL24 of the stromal cells appear faint and less positive in tubular adenoma cases with low-grade dysplasia (G, CCL11; J, CCL24), high-grade dysplasia (H, CCL11; K, CCL24), and adenocarcinoma (I, CCL11; L, CCL24).

and CCL24 with the degree of eosinophilia revealed that tumoral expression of CCL11 and CCL24 decreased and stromal expression of CCL11 and CCL24 increased while the number of tissue-infiltrating eosinophils decreased (both comparisons, $p < .001$) (Table 2).

## DISCUSSION

Our study showed that the number of tissue infiltrating eosinophils in colorectal neoplasms decreased significantly in colorectal adenocarcinoma cases compared to tubular adenoma cases with low-grade dysplasia and tubular adenoma cases with high-grade dysplasia, which is consistent with the results of pre-


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**Table 1.** Expression of CCL11 and CCL24 in the tumor and stromal cells according to tumor progression

| Variable | Tumor | | | | | Stroma | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Low | Intermediate | High | No. | p-value | Low | Intermediate | High | No. | p-value |
| CCL11 expression | | | | | | | | | | |
| Low-grade dysplasia | 10 (20.4) | 25 (51.0) | 14 (28.6) | 49 | <.001 | 37 (75.5) | 12 (24.5) | 0 (0) | 49 | <.001 |
| High-grade dysplasia | 26 (56.5) | 19 (41.3) | 1 (2.1) | 46 | | 20 (38.5) | 26 (50.0) | 0 (0) | 46 | |
| Adenocarcinoma | 39 (82.9) | 8 (17.0) | 0 (0) | 47 | | 7 (15.2) | 32 (69.6) | 7 (15.2) | 46 | |
| CCL24 expression | | | | | | | | | | |
| Low-grade dysplasia | 3 (6.1) | 23 (46.9) | 23 (46.9) | 49 | <.001 | 30 (61.2) | 19 (38.8) | 0 (0) | 49 | <.001 |
| High-grade dysplasia | 36 (78.2) | 10 (21.7) | 0 (0) | 46 | | 28 (60.8) | 17 (37.0) | 1 (2.1) | 46 | |
| Adenocarcinoma | 41 (83.6) | 8 (17.0) | 0 (0) | 49 | | 5 (10.4) | 33 (68.8) | 10 (20.8) | 48 | |

Values are presented as number (%).

**Table 2.** Expression of CCL11 and CCL24 in the tumor and stromal cells according to eosinophilia grading

| Variable | Tumor | | | | | Stroma | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Low | Intermediate | High | No. | p-value | Low | Intermediate | High | No. | p-value |
| CCL11 expression | | | | | | | | | | |
| Eosinophils | | | | | | | | | | |
| Low | 48 (68.6) | 19 (27.1) | 3 (4.3) | 70 | <.001 | 20 (29.0) | 43 (62.3) | 6 (8.7) | 69 | .001 |
| Moderate | 23 (43.4) | 19 (35.8) | 11 (20.8) | 53 | | 35 (66.0) | 17 (32.1) | 1 (1.9) | 53 | |
| High | 4 (21.1) | 14 (73.7) | 1 (5.3) | 19 | | 9 (47.4) | 10 (52.6) | 0 (0) | 19 | |
| CCL24 expression | | | | | | | | | | |
| Eosinophils | | | | | | | | | | |
| Low | 56 (78.9) | 12 (16.9) | 3 (4.2) | 71 | <.001 | 20 (28.2) | 40 (56.3) | 11 (15.5) | 71 | <.001 |
| Moderate | 17 (32.1) | 22 (41.5) | 14 (26.4) | 53 | | 31 (58.5) | 22 (41.5) | 0 (0) | 53 | |
| High | 6 (31.6) | 7 (36.8) | 6 (31.6) | 19 | | 12 (63.2) | 7 (36.8) | 0 (0) | 19 | |

Values are presented as number (%).

vious studies.[17] Colorectal cancer is well known as a non-immunogenic tumor that induces an impaired immune response to the tumor itself, thus evading the host immune response to cancer.[29-31] It is also known that an increase in tissue-infiltrating eosinophils in colorectal cancer is associated with a better prognosis. Therefore, decreased tissue eosinophilia may be an immune-evading strategy of colon cancer. Because eosinophils develop and migrate to tissues in response to chemokine signaling, chemokine expression in colorectal cancer should reveal the eosinophil-infiltration potential of colorectal cancer in individual cases.

In colorectal cancer patients, both the serum level and concentration of CCL11 in tumor tissue are elevated.[24,29,30] Since CCL11 enhances tissue recruitment of eosinophils, an increased concentration of CCL11 should attract more eosinophils and cause more severe eosinophilia in tissues where the concentration of CCL11 is high. Indeed, a study on eosinophilia in colorectal neoplastic lesions showed that tissue extracted from a tumor with a greater number of eosinophils was also highly chemotactic for eosinophils, which was thought to reflect chemokine concentrations in the tissue.[19,23] Another study involving immunohistochemical staining of colorectal cancer tissue revealed that expression of CCL11 was elevated in stromal cells, such as fibroblasts or lymphocytes.[24] The results of this study are consistent with our data showing that expression of CCL11 and CCL24 is increased in the stromal cells of adenocarcinomas compared to those that are dysplastic. The CCL11/CCL24-secreting stromal cells in our study were mostly mononuclear inflammatory cells. Increased expression of CCL11 and CCL24 in the stromal cells of tumors might explain the elevated serum chemokine levels and elevated tissue concentration of chemokines. However, previous studies on eosinophilia in colorectal neoplastic lesions reported decreased numbers of eosinophils in tissues with adenocarcinoma or high-grade dysplasia,[17,19] which is consistent with our results. Thus the question remains: why is decreased eosinophilia observed in association with colorectal malignant neoplastic lesions even when the concentration of chemokines for eosinophils is elevated?

Our results showed that CCL11 and CCL24 expression was lower in glandular cells of adenocarcinomas compared to the expression levels in stromal cells. These findings might provide insight into the discrepancy between tissue eosinophilia and tissue chemokine concentrations in colorectal neoplasms. As we

previously mentioned, the population of tissue-infiltrating eosinophils is lower in colorectal adenocarcinomas, as is the expression of CCL11 and CCL24 in neoplastic glandular cells of adenocarcinomas. However, one study found increased tissue concentration of CCL11 associated with colorectal adenocarcinoma.[23] We postulate that increased expression of CCL11 and CCL24 in the stromal cells of tumors might explain increased tissue CCL11 concentration. If lower expression of CCL11 and CCL24 in neoplastic glandular cells is responsible for decreased eosinophilia in adenocarcinomas, modulation of chemokine expression could contribute to the immune-evasion mechanisms of colorectal adenocarcinomas by inhibiting recruitment of eosinophils, which function as effector cells for the neoplasm.

Further studies on other chemokines involved in eosinophil physiology and studies on the status of eosinophils recruited to tumor tissues are needed for a more detailed understanding of the nature of peritumoral eosinophilia and its significance for the immunologic characteristics of colorectal cancer. CCL24 has previously been a target of anti-cancer immune therapies.[23] If reduced expression of chemokines contributes to immune evasion by colorectal cancer, modulation of chemokine expression in cancer cells could be a possible target for anticancer therapies as well as a prognostic factor for colorectal cancer.[31,32] As some studies on leukemia and CCL24 have suggested, specific kinds of chemokines might affect the migration of specific types of eosinophils in colorectal cancer.[33]

In conclusion, we found a significant correlation between eosinophil numbers and immunohistochemical staining of CCL11 and CCL24 chemokines in the glandular cells of colorectal neoplasms. Lower expression of CCL11 and CCL24 was observed in tumor glandular cells, while greater expression was observed in tumor stromal cells. This differential expression of chemokines might help explain the decreased eosinophilia observed in colorectal cancer despite an apparent increased concentration of CCL11, and could provide insight into immune evasion mechanisms of colorectal cancer. Considering that eosinophils are antitumoral effector immune cells, cancer appears to induce a decrease in eosinophilia through decreased expression of chemokines in glandular cells, which is consistent with the decreased expression of CCL11 and CCL24 shown in our study, and thus, achieves immune evasion. Further study on eosinophil-associated chemokines and the nature of the eosinophils recruited by colorectal cancer cells might enhance our understanding of the immunologic characteristics and roles of eosinophils in colorectal cancer.

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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■ ORIGINAL ARTICLE ■

# Immunohistochemical Expression and Clinical Significance of Suggested Stem Cell Markers in Hepatocellular Carcinoma

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**Background:** Increasing evidence has shown that tumor initiation and growth are nourished by a small subpopulation of cancer stem cells (CSCs) within the tumor mass. CSCs are posited to be responsible for tumor maintenance, growth, distant metastasis, and relapse after curative operation. We examined the expression of CSC markers in paraffin-embedded tissue sections of hepatocellular carcinoma (HCC) and correlated the results with clinicopathologic characteristics. **Methods:** Immunohistochemical staining for the markers believed to be expressed in the CSCs, including epithelial cell adhesion molecule (EpCAM), keratin 19 (K19), CD133, and CD56, was performed in 82 HCC specimens. **Results:** EpCAM expression was observed in 56% of the HCCs (46/82) and K19 in 6% (5/82). EpCAM expression in HCC significantly correlated with elevated α-fetoprotein level, microvessel invasion of tumor cells, and high histologic grade. In addition, EpCAM expression significantly correlated with K19 expression. The overall survival and relapse-free survival rates in patients with EpCAM-expressing HCC were relatively lower than those in patients with EpCAM-negative HCC. All but two of the 82 HCCs were negative for CD133 and CD56, respectively. **Conclusions:** Our results suggest that HCCs expressing EpCAM are associated with unfavorable prognostic factors and have a more aggressive clinical course than those not expressing EpCAM. Further, the expression of either CD133 or CD56 in paraffin-embedded HCC tissues appears to be rare.

**Key Words:** Carcinoma, hepatocellular; EpCAM protein; Cancer stem cells

Hepatocellular carcinoma (HCC) is recognized worldwide as the fifth most common solid cancer and the third leading cause of cancer-associated mortality.[1] Although remarkable advancement in the treatment of HCC has been made over the past few decades, HCC is still related to a high rate of mortality because of its recurrence and metastasis.[2] Increasing evidence has demonstrated that tumor maintenance and growth are nourished by a small subpopulation of cancer stem cells (CSCs) within the tumor mass. CSCs are hypothesized to be responsible for tumor initiation, recurrence, generation of distant metastases, and resistance to radiation and chemotherapy.[3-7] Recent research on HCC has centered on the issues of CSCs, which include identification of CSCs, expansion of CSC markers, and therapeutic targeting of CSCs,[7-11] since CSC markers might be useful for estimating the prognosis of HCC patients. Several markers including epithelial cell adhesion molecule (EpCAM),[8] keratin 19 (K19),[9] CD133,[10] and CD56[11] have been proposed as CSC markers in HCC, and they are termed stemness markers.

In the present study, the location and expression of four suggested CSC markers, namely EpCAM, K19, CD133, and CD56, were examined in paraffin-embedded tissue sections of 82 HCCs, and the relationships between expression of these markers and clinicopathologic characteristics of HCC were investigated.

## MATERIALS AND METHODS

### Patients and specimens

This study was approved by the Institutional Review Board (IRB) of Chonbuk National University Hospital. Informed consent was obtained from all patients who underwent surgery according to the Helsinki Declaration. To examine the location and expression of four suggested CSC markers, we collected 91 surgical specimens of formalin-fixed, paraffin-embedded HCCs resected in the Department of Pathology, Chonbuk National University Hospital, between January 2011 and December 2013. Among these patients, nine underwent transarterial chemoem-





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bolization, which resulted in near total necrosis of the tissue. The remaining 82 patients were analyzed in our study. In each case, clinicopathologic findings, including age, gender, etiology, background liver disease, ascites, serological data including serum albumin level and α-fetoprotein (AFP) level, microvessel invasion, intrahepatic metastasis, histologic grade, and follow-up data, were obtained from hospital records. Tumors were staged according to the criteria of the 2010 American Joint Committee on Cancer (AJCC) TNM classification.[12] Follow-up period was defined as the period from the date of initial surgery to the date of either last follow-up or death.

### Immunohistochemistry

For immunohistochemical (IHC) staining, 10% formalin-fixed, paraffin-embedded tissue sections of representative areas of tumor were prepared into 4-μm-thick tissue samples. IHC staining was performed using a fully automated IHC system with the Bond Polymer Refine Detection Kit (Leica Bond, Newcastle upon Tyne, UK) according to the manufacturer's instructions. The sources of antibodies used in this study and conditions of the procedure are listed in Table 1. Peroxidase activity was detected with the enzyme substrate 3-amino-9-ethyl carbazole. The immunoreactivity of the specimens was interpreted according to the intensity of staining and proportion of positive cells. The intensity of cytoplasmic and membranous staining was graded into four levels: no immunostaining (0), weak (1), moderate (2), and intense (3). The proportion of positive cells was scored as follows: 0 (none), 1 (1%), 2 (2%–10%), 3 (11%–33%), 4 (34%–66%), and 5 (67%–100%). The sum index was obtained by totaling the scores of intensity and proportion of staining. If the final score was equal to or greater than 4, the immunoreactivity was considered positive; otherwise, the immunoreactivity was considered negative. For negative controls, sections were treated in the same way, except that they were incubated with Tris-buffered saline instead of primary antibodies.

### Statistical analysis

SPSS ver. 15.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Associations between the clinicopathological factors and expression of antibodies were tested using a chi-square test. Univariate and multivariate Cox proportional regression analyses for overall survival and relapse-free survival were performed. A p-value of $< .05$ was considered statistically significant.

## RESULTS

### Clinical features

The 82 patients included 72 males and 10 females with a mean age of 57 years (range, 26 to 77 years). HCC formation was attributed to the presence of hepatitis B virus (HBV) surface antigen in 64 patients, alcohol-related complication in seven patients, presence of anti–hepatitis C virus antibody in four patients, and unknown etiology in seven patients. The surrounding liver tissue showed cirrhosis in 56% of the patients (46/82) and chronic hepatitis with varying degrees of fibrosis in the remaining 44% of the patients (36/82).

### Expression of suggested CSC markers

In cirrhotic livers, all four examined markers showed intense immunoreactivity in proliferating reactive bile ductules. These ductules are thought to originate from hepatic progenitor cells and thus were considered as an internal positive control. EpCAM expression in HCC tissues showed intense cytoplasmic and/or membranous staining (Fig. 1). In 82 HCC samples, 46 were EpCAM-positive (56%) and five were K19-positive (6%). Of the 82 HCCs, only two cases showed CD133 and CD56 expression, respectively.

### Correlations between expression of suggested CSC markers and clinicopathological features

Analysis of the relationships between expression of K19, CD133, and CD56 and clinicopathologic features was not meaningful, since the number of cases with K19, CD133, and CD56 expression was very small. However, EpCAM expression significantly correlated with higher histologic grade ($p = .006$), elevated AFP level ($p = .001$), and microvessel invasion of the tumor cells ($p = .030$) (Table 2). Additionally, EpCAM expression was

**Table 1.** Summary of antibodies and conditions used for immunohistochemistry

| Antibody | Clone | Source | Dilution | Antigen retrieval | Antibody incubation |
|---|---|---|---|---|---|
| EpCAM | VU-1D9 | Calbiochem | 1:500 | BOND ER solution I (pH 6.0), 100°C, 20 min | Room temperature, 20 min |
| K19 | BA17 | DakoCytomation | 1:100 | BOND ER solution I (pH 6.0), 100°C, 20 min | Room temperature, 20 min |
| CD133 | AC133 | Miltenyi Biotec | 1:50 | BOND ER solution II (pH 9.0), 100°C, 20 min | Room temperature, 20 min |
| CD56 | 123C3 | Zymed Laboratories | 1:400 | BOND ER solution II (pH 9.0), 100°C, 20 min | Room temperature, 20 min |

EpCAM, epithelial cell adhesion molecule; K19, keratin 19.

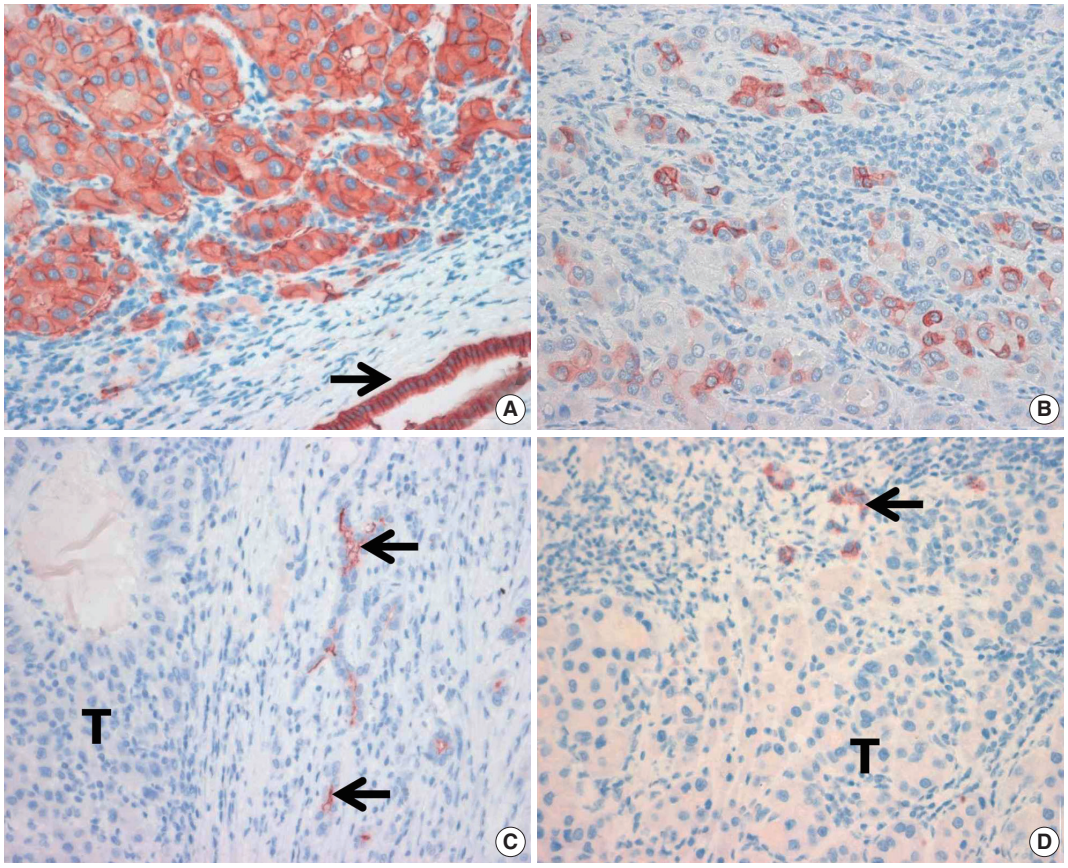


**Fig. 1.** (A) Membranous expression of epithelial cell adhesion molecule (EpCAM) in hepatocellular carcinoma cells. Bile duct epithelial cells also showed strong expression of EpCAM (arrow). (B) Tumor cells with cytoplasmic reactivity for keratin 19. Absence of CD133 (C) and CD56 (D) immunoreactivity in tumor cells (T). Note the positive immunoreactivity for CD133 and CD56 in bile ductular cells (arrows).

strongly associated with K19 expression (p = .041).

### Outcome

In 82 patients with HCC, follow-up intervals ranged from 0.1 to 69 months (mean of 23 months). Eleven patients died, and 16 showed local recurrence or latent distant metastasis. The mean overall survival time of patients with EpCAM-expressing HCC was 57.2 ± 3.7 months, while that of EpCAM-negative HCC was 62.0 ± 2.9 months. The mean relapse-free survival time of patients with EpCAM-expressing HCC was 40.5 ± 5.9 months, while that of patients with EpCAM-negative HCC was 53.8 ± 5.0 months. However, these results were not statistically significant. In univariate Cox regression analysis, T category significantly correlated with poor patient overall survival (p = .003). Additionally, T category, preoperative serum AFP level, and vascular invasion were associated with decreased relapse-free survival (p = .019, p = .048, and p = .011, respectively). Multivariate analysis revealed that T category and vascular invasion were independent indicators of relapse (p = .011 and p = .007, respectively).

## DISCUSSION

Carcinogenesis is currently explained using two models, one involving a traditional stochastic onset and a second based on the role of CSCs. According to the CSC model, only a small population of tumor cells has the ability to divide and repopulate within the tumor.[7,13] CSCs are responsible for tumor initiation, maintenance, growth, metastasis, and relapse after therapy.[3-6] The expression of several proposed CSC markers in HCC has been reported and might prove to be useful for predicting the prognosis of HCC patients.[11,14-16] However, little is known about the relationships between the IHC expression of CSC markers in paraffin-embedded tissue sections and clinicopathologic factors or clinical outcomes of HCC.

EpCAM is a transmembrane intercellular protein that was initially posed as a homophilic cell adhesion molecule[17] and is highly up-regulated in most human epithelial cancers, including HCC.[14-16,18] EpCAM has been validated as a marker of stem cells in the liver, and EpCAM-positive HCC is likely to have


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**Table 2.** The relationship between the expression of suggested stem cell marker EpCAM and clinicopathologic characteristics

| Characteristic | | No. | EpCAM | | |
|---|---|---|---|---|---|
| | | | Positive | Negative | p-value |
| Age (yr) | <60 | 49 | 30 | 19 | .254 |
| | ≥60 | 33 | 16 | 17 | |
| Sex | Female | 10 | 7 | 3 | .344 |
| | Male | 72 | 39 | 33 | |
| AFP (ng/mL) | <100 | 58 | 26 | 32 | .001 |
| | ≥100 | 24 | 20 | 4 | |
| Albumin (g/dL) | ≥3.5 | 79 | 45 | 34 | .418 |
| | <3.5 | 3 | 1 | 2 | |
| PIVKA-II (mAU/mL) | <50 | 26 | 25 | 1 | .798 |
| | ≥50 | 44 | 24 | 20 | |
| T category | I | 34 | 16 | 18 | .230 |
| | II | 36 | 24 | 12 | |
| | III and IV | 12 | 6 | 6 | |
| Cirrhosis | Absence | 36 | 24 | 12 | .088 |
| | Presence | 46 | 22 | 24 | |
| Etiology | Non-viral | 14 | 7 | 7 | .614 |
| | Viral | 68 | 39 | 29 | |
| Microvessel invasion | Absence | 39 | 17 | 22 | .030 |
| | Presence | 43 | 29 | 14 | |
| Intrahepatic metastasis | Absence | 67 | 36 | 31 | .362 |
| | Presence | 15 | 10 | 5 | |
| Ascites | Absence | 75 | 44 | 31 | .125 |
| | Presence | 7 | 2 | 5 | |
| Histologic grade | 1 and 2 | 50 | 22 | 28 | .006 |
| | 3 and 4 | 32 | 24 | 8 | |
| K19 | Negative | 77 | 41 | 36 | .041 |
| | Positive | 5 | 5 | 0 | |

EpCAM, epithelial cell adhesion molecule; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence/antagonist-II; K19, keratin 19.

originated from hepatic stem cells.[8,15,16,19] In the present study, we observed that EpCAM expression significantly correlated with tumor progression factors of HCC, such as elevated AFP level, vascular invasion, and high tumor grade. These findings are in agreement with previous studies showing that expression level of EpCAM correlates with de-differentiation[11] and vascular invasion and is associated with the high AFP level in HCC.[20,21] The prognosis of patients with EpCAM-negative HCC is considered to be better than that of those with EpCAM-positive HCC.[11,14-16,20,21] Although EpCAM expression was not found to be an independent predictor of survival in patients with HCC in this study, EpCAM expression was found to be associated with well-known unfavorable prognostic factors in HCC. Thus, the use of EpCAM expression as an unfavorable prognostic factor of HCC is reasonable. Furthermore, gene expression profile study has demonstrated that EpCAM-positive and AFP-positive HCCs have more aggressive behavior and poor clinical outcome, whereas EpCAM-negative and AFP-negative HCCs have good prognosis.[20] Based on these observations, EpCAM has attracted considerable attention as a possible therapeutic target for patients with HCC.[7,8,13] Taken together, our findings suggest that EpCAM is a critical player in facilitating vascular invasion of tumor cells, leading to de-differentiation in HCC with high serum AFP level, and might be useful for predicting the prognosis of HCC patients. In this study, a proportion of HCCs (56%) expressed EpCAM, and EpCAM expression was associated with K19 expression. These findings are in agreement with previous reports showing that the expression of EpCAM and K19 was positively correlated.[15,21] The proportion of tissues expressing EpCAM in this study was a little higher than in earlier series, which reported EpCAM in 15.9%–48.7% of HCCs.[15-17,20-23] Kimura *et al*.[23] have demonstrated that EpCAM expression was observed more often in HCC patients with HBV than in those with other etiologies, and the immunoreactivity of EpCAM has been found in up to 78% of HCC patients with HBV. The high proportion of HCC patients with HBV (74%) examined in this study might be a possible explanation for the higher EpCAM-positive rate. It has been hypothesized that the transformation

of hepatic stem/progenitor cells (maturation arrest theory) underlies the occurrence of HCC expressing CSC markers. However, the concept of the CSC model is still debated. The expression of CSC markers in HCC can develop as an acquisition of progenitor cell features during the de-differentiation of cancer cells (de-differentiation theory).[5,15] Kim and Park[24] have proposed that HCC expressing CSC markers should be designated as "HCC with stemness-related marker expression" in order to avoid the implication that these HCCs originated from hepatic stem/progenitor cells.

Either CD133 or CD56 expression in this study was rare. Only two of 82 HCC specimens showed positive staining for CD133 and CD56, suggesting that CD133 and CD56 expression is rare in paraffin-embedded HCC tissues. It has been reported that 0%–88%[11,15,16,25] of human HCC tissue samples express CD133, and the expression of which is negatively correlated with presence of HBV.[26] Several IHC studies have demonstrated CD56 expression in 0%–9.7% of human HCCs.[11,25,27,28] The reason for this high discrepancy of positive rate of CD133 and CD56 in HCC is unclear. In our study, we employed the bond polymer IHC staining method to avoid endogenous biotin contamination, and all four examined markers always showed strong immunoreactivity in reactive bile ductules, which were considered as internal positive controls. The discrepancy in the expression rates of CD133 and CD56 is not easily explained but might be related to the different criteria for positivity, the quality of tissue samples analyzed, and the unique antibodies and immunostaining methods used in different studies. Our negative results in analysis of CD133 and CD56 through the use of specific antibodies in an established automated IHC system does not exclude the presence of these markers and can be linked to the different etiologies according to geographic background. Additional investigations with a larger population of HCC and strict criteria for positive immunoreactivity are necessary to determine the expression and clinical implication of these markers.

In conclusion, we showed that the EpCAM expression in paraffin-embedded tissue sections of HCC is associated with tumor progression factors and might be useful for predicting the prognosis of HCC patients. The high proportion of EpCAM expression in HCCs and its associations with unfavorable prognostic factors of HCC provide a basis for further investigation of anti-EpCAM–targeted therapy.

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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# Evaluation of the VE1 Antibody in Thyroid Cytology Using *Ex Vivo* Papillary Thyroid Carcinoma Specimens

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**Background:** Recently, VE1, a monoclonal antibody against the *BRAF*V600E mutant protein, has been investigated in terms of its detection of the *BRAF*V600E mutation. Although VE1 immunostaining and molecular methods used to assess papillary thyroid carcinoma in surgical specimens are in good agreement, evaluation of VE1 in thyroid cytology samples is rarely performed, and its diagnostic value in cytology has not been well established. In present study, we explored VE1 immunoexpression in cytology samples from ex vivo papillary thyroid carcinoma specimens in order to minimize limitations of low cellularity and sampling/targeting errors originated from thyroid fine-needle aspiration and compared our results with those obtained using the corresponding papillary thyroid carcinoma tissues. **Methods:** The VE1 antibody was evaluated in 21 cases of thyroid cytology obtained directly from *ex vivo* thyroid specimens. VE1 immunostaining was performed using liquid-based cytology, and the results were compared with those obtained using the corresponding tissues. **Results:** Of 21 cases, 19 classic papillary thyroid carcinomas had *BRAF*V600E mutations, whereas two follicular variants expressed wild-type *BRAF*. VE1 immunoexpression varied according to specimen type. In detection of the *BRAF*V600E mutation, VE1 immunostaining of the surgical specimen exhibited 100% sensitivity and 100% specificity, whereas VE1 immunostaining of the cytology specimen exhibited only 94.7% sensitivity and 0% specificity. **Conclusions:** Our data suggest that VE1 immunostaining of a cytology specimen is less specific than that of a surgical specimen for detection of the *BRAF*V600E mutation, and that VE1 immunostaining of a cytology specimen should be further evaluated and optimized for clinical use.

**Key Words:** Thyroid gland; Biopsy, fine-needle; Cytology; *BRAF* mutation; Immunohistochemistry

BRAF, a serine/threonine kinase and the v-RAF murine sarcoma viral oncogene homolog B1, is an activator of the mitogen-activated protein kinase (MAPK) pathway.[1] Mutations in *BRAF* constitutively activate the MAPK pathway, allowing human cancers to develop and progress.[1,2] Of the various *BRAF* mutations, *BRAF*V600E, a valine to glutamic acid substitution at codon 600, is the most common.[1,3] In clinical practice, the *BRAF*V600E mutation is of major interest because it is considered a critical diagnostic, prognostic, and predictive biomarker of many types of cancer.[3-6] Among the many endocrine malignancies, the *BRAF*V600E mutation is a reliable diagnostic marker of papillary thyroid carcinoma (PTC), as it is detected in 40%–80% of PTCs but virtually never in benign tumors.[3,7] Currently, the *BRAF*V600E mutation in PTC is typically identified using DNA-based methods such as direct sequencing, allele-specific polymerase chain reaction (PCR), or real-time PCR.[7,8] Although these methods all afford high sensitivity and specificity, expensive equipment and rigorous quality control are required.[8-10]

Recently, the VE1 antibody, a monoclonal antibody against the *BRAF*V600E mutant protein, was investigated in terms of its detection of the *BRAF*V600E mutation.[9] Although VE1 immunostaining revealed a high concordance rate with molecular methods in surgical specimens of PTC,[10-12] evaluation of VE1 in thyroid cytology samples is rarely performed, and its diagnostic value in cytology has not been well established.[13-16] In the present study, we evaluated the use of the VE1 antibody in cytology samples from *ex vivo* thyroid PTC specimens in order to overcome the drawbacks of fine-needle aspiration (FNA) including low cellularity and sampling/targeting errors,[13,15,16] and the results were compared to the data from corresponding PTC tissues.

## MATERIALS AND METHODS

This study was approved by the Ajou University Hospital In-





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stitutional Review Board (AJIRB-BMR-OBS-13-342). Cytology samples were obtained from fresh *ex vivo* PTC tissues immediately following surgical resection in cases that provided informed consent. After gross examination of fresh PTC specimens, cytology samples were obtained by scraping representative cancerous areas. Smear slides were prepared and stained with hematoxylin and eosin to explore the adequacy of liquid-based cytology (LBC). In later evaluations, LBC slides were prepared using the BD SurePath method employing CytoRich Red (TriPath Inc., Burlington, NC, USA). PTC tissues were fixed in 4% buffered formalin and, after embedding in paraffin, processed for histology and ancillary tests.

### Immunohistochemistry and immunocytochemistry

Formalin-fixed, paraffin-embedded tissue blocks that included the cytology-sampled lesion were sectioned at a 4-μm slice thickness and deparaffinized for immunohistochemistry (IHC). VE1 immunostaining was performed using the aid of a Benchmark XT automated IHC platform (Ventana Medical Systems, Tucson, AZ, USA), as described previously.[16] Briefly, after cell conditioning (conditioner 1) for 64 minutes and inhibition of the preprimary peroxidase, slides were incubated with the VE1 antibody (1:50, Spring Bioscience, Pleasant, CA, USA) at 37°C for 32 minutes. Primary antibodies were detected using an OptiView DAB IHC Detection kit (Ventana Medical Systems) following incubation with hematoxylin and a bluing reagent (4 minutes each). For immunocytochemistry (ICC), unstained LBC slides were fixed in 95% ethyl alcohol for a minimum of 30 minutes. The ICC protocol was identical to that of IHC, except that the cells were not conditioned.

Two pathologists (J.-H.K. and Y.H.K), blinded to the molecular findings, assessed all IHC and ICC data independently; any difference in the interpretation was resolved by consensus. The extent of VE1 staining was graded from 0 to 3: 0, negative; 1, VE1 staining in < 30% of cells; 2, VE1 staining in 30%–80% of cells; and 3, VE1 staining in > 80% of cells. In terms of cytoplasmic staining of follicular cells, intensity was also graded from 0 to 3: 0, negative; 1, weak; 2, moderate; and 3, strong. In defining *BRAF*V600E mutation, VE1 immunostaining was considered positive if the intensity of cytoplasmic staining was grade 2 or 3, regardless of the overall extent of staining.[13,16]

In cases of discrepancy between immunostaining and molecular results, we repeated immunostaining with a different method, the Ultravision LP Detection System (Thermo Fisher Scientific, Fremont, CA, USA), and re-evaluated the results.

### Detection of the *BRAF*V600E mutation

For genomic DNA isolation, formalin-fixed, paraffin-embedded tissue blocks were sectioned at 10-μm thickness. Genomic DNA was extracted from manually microdissected tumor areas from each tissue section using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To detect the *BRAF*V600E mutation, mutant enrichments with 3'-modified oligonucleotide sequencing were performed to confirm the presence or absence of the *BRAF*V600E mutation, employing primers and PCR conditions as described previously.[7] Results were analyzed using Sequencher 4.10 software (Gene Codes, Ann Arbor, MI, USA).

In cases of discrepancy between immunostaining and molecular results, we repeated molecular testing with a different method, the PNAClamp Technology (Panagene, Daejeon, Korea), and re-evaluated the results.

## RESULTS

VE1 immunostaining of LBC material and formalin-fixed, paraffin-embedded tissue sections of the corresponding areas was performed in 21 *ex vivo* PTC specimens. Clinicopathological characteristics of the 21 cases are summarized in Table 1. Of these, 19 were classic PTC cases, and two were follicular variants of PTC. The results of VE1 immunostaining according to *BRAF*V600E mutation status are shown in Table 2. Of the 21 cases, VE1 IHC of the 19 classic PTC cases exhibited diffuse immunoexpression with moderate or strong intensity, whereas staining in the two follicular variants was weak. Upon VE1 ICC, however, only 11 PTC cases (52.4%) exhibited diffuse immunoexpression (Table 3). The remaining cases yielded focal (2 cases, 9.5%) or multifocal (8 cases, 38.1%) immunostaining patterns (Fig. 1). Of the 21 cases with VE1 ICC, only 11 (52.4%) exhibited immunostaining intensity as strong as that of the corresponding VE1 IHC staining. In six cases (28.6%), immunostaining intensity was weaker than VE1 IHC staining, and in four cases (19.4%), VE1 immunostaining intensity was stronger than VE1 IHC staining (Table 4). VE1 immunostaining was interpreted as positive in 19 IHC and 20 ICC specimens (Table 5). We varied the molecular and immunohistochemical methods in cases of discrepancy between VE1 immunostaining and molecular results, but the results were similar (Appendices 1–3). In terms of the *BRAF*V600E mutation, VE1 immunostaining exhibited 100% sensitivity and 100% specificity with IHC but 94.7% sensitivity and 0% specificity with ICC.

## DISCUSSION

Clinical applications of VE1 immunostaining in terms of thyroid cytology evaluation are of great interest because PTC diagnosis in daily clinical practice is generally based on thyroid FNA cytology; immunostaining is simple, inexpensive, and routinely

**Table 1.** Clinicopathological characteristics of 21 cases of papillary thyroid carcinoma

| Case No. | Sex | Age (yr) | Histological type | Tumor diameter (cm) | T stage | N stage | *BRAF*V600E mutation |
|---|---|---|---|---|---|---|---|
| 1 | F | 49 | Classic | 1 | T3 | N1a | Present |
| 2 | F | 51 | Classic | 1.2 | T3 | N0 | Present |
| 3 | M | 43 | Classic | 0.7 | T3 | N1a | Present |
| 4 | F | 30 | Classic | 0.9 | T3 | N0 | Present |
| 5 | F | 35 | Classic | 1.2 | T3 | N1a | Present |
| 6 | M | 48 | Classic | 0.9 | T1a | N0 | Present |
| 7 | F | 30 | Classic | 1.2 | T3 | N1a | Present |
| 8 | M | 26 | FVPTC | 1.4 | T3 | N1a | Absent |
| 9 | F | 60 | Classic | 1.8 | T3 | N1a | Present |
| 10 | F | 56 | Classic | 1 | T3 | N1a | Present |
| 11 | F | 63 | Classic | 0.8 | T3 | N1b | Present |
| 12 | M | 69 | Classic | 1.3 | T3 | N1a | Present |
| 13 | F | 48 | Classic | 0.8 | T3 | N0 | Present |
| 14 | F | 59 | Classic | 1 | T3 | N0 | Present |
| 15 | F | 46 | Classic | 1.5 | T1b | N1a | Present |
| 16 | F | 62 | Classic | 1.2 | T1b | N0 | Present |
| 17 | F | 54 | Classic | 1 | T3 | N0 | Present |
| 18 | F | 63 | Classic | 3.3 | T3 | N1a | Present |
| 19 | F | 42 | Classic | 0.7 | T1a | N1a | Present |
| 20 | M | 69 | Classic | 0.8 | T1a | N0 | Present |
| 21 | F | 52 | FVPTC | 1.2 | T3 | N0 | Absent |

FVPTC, follicular variant papillary thyroid carcinoma.

**Table 2.** VE1 immunoexpression and mutation status (*BRAF*V600E) in 21 cases of papillary thyroid carcinoma

| Case No. | Liquid-based cytology | | Histology | | *BRAF*V600E mutation |
|---|---|---|---|---|---|
| | Distribution | Intensity | Distribution | Intensity | |
| 1 | 2+ | 3+ | 3+ | 3+ | Present |
| 2 | 2+ | 2+ | 3+ | 3+ | Present |
| 3 | 1+ | 1+ | 3+ | 3+ | Present |
| 4 | 2+ | 2+ | 3+ | 3+ | Present |
| 5 | 3+ | 3+ | 3+ | 3+ | Present |
| 6 | 1+ | 2+ | 3+ | 2+ | Present |
| 7 | 2+ | 3+ | 3+ | 3+ | Present |
| 8 | 2+ | 2+ | 3+ | 1+ | Absent |
| 9 | 2+ | 3+ | 3+ | 3+ | Present |
| 10 | 2+ | 2+ | 3+ | 3+ | Present |
| 11 | 3+ | 3+ | 3+ | 2+ | Present |
| 12 | 3+ | 3+ | 3+ | 2+ | Present |
| 13 | 3+ | 3+ | 3+ | 3+ | Present |
| 14 | 3+ | 3+ | 3+ | 3+ | Present |
| 15 | 3+ | 3+ | 3+ | 3+ | Present |
| 16 | 3+ | 3+ | 3+ | 3+ | Present |
| 17 | 3+ | 3+ | 3+ | 3+ | Present |
| 18 | 3+ | 3+ | 3+ | 3+ | Present |
| 19 | 3+ | 2+ | 3+ | 3+ | Present |
| 20 | 3+ | 2+ | 3+ | 3+ | Present |
| 21 | 2+ | 2+ | 3+ | 1+ | Absent |

Distributions of VE1-positive cells: 0+, 0%; 1+, <30%; 2+, 30%–80%; and 3+, >80%. Intensities of VE1-positive cells: 0+, none; 1+, weak; 2+, moderate; and 3+, strong.


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performed in most pathology laboratories. Moreover, VE1 immunostaining is not dependent on DNA quality or the proportion of tumor cells in a FNA sample and allows for *in situ* assessment of tumor cells expressing the *BRAF*V600E mutant protein at a single-cell level.[13,16]

In the present study, we evaluated the VE1 antibody in thyroid cytology using LBC specimens obtained directly from surgical-

**Table 3.** Distribution of VE1 expression evaluated via ICC and IHC

| Distribution of VE1 | ICC | | |
|---|---|---|---|
| | <30% | 30–80% | >80% |
| IHC | | | |
| <30% | - | - | - |
| 30%–80% | - | - | - |
| >80% | 2 | 8 | 11 |

ICC, immunocytochemistry; IHC, immunohistochemistry.

**Table 4.** Intensities of VE1 expression evaluated via ICC and IHC

| Intensity of VE1 | ICC | | |
|---|---|---|---|
| | Weak | Moderate | Strong |
| IHC | | | |
| Weak | - | 2 | - |
| Moderate | - | 1 | 2 |
| Strong | 1 | 5 | 10 |

ICC, immunocytochemistry; IHC, immunohistochemistry.

**Table 5.** Comparison between *BRAF*V600E mutation and VE1 expression statuses evaluated via ICC and IHC

| *BRAF*V600E mutation | VE1 ICC | | VE1 IHC | |
|---|---|---|---|---|
| | Negative | Positive | Negative | Positive |
| Absence | 0 | 2 | 2 | 0 |
| Presence | 1 | 18 | 0 | 19 |

ICC, immunocytochemistry; IHC, immunohistochemistry.

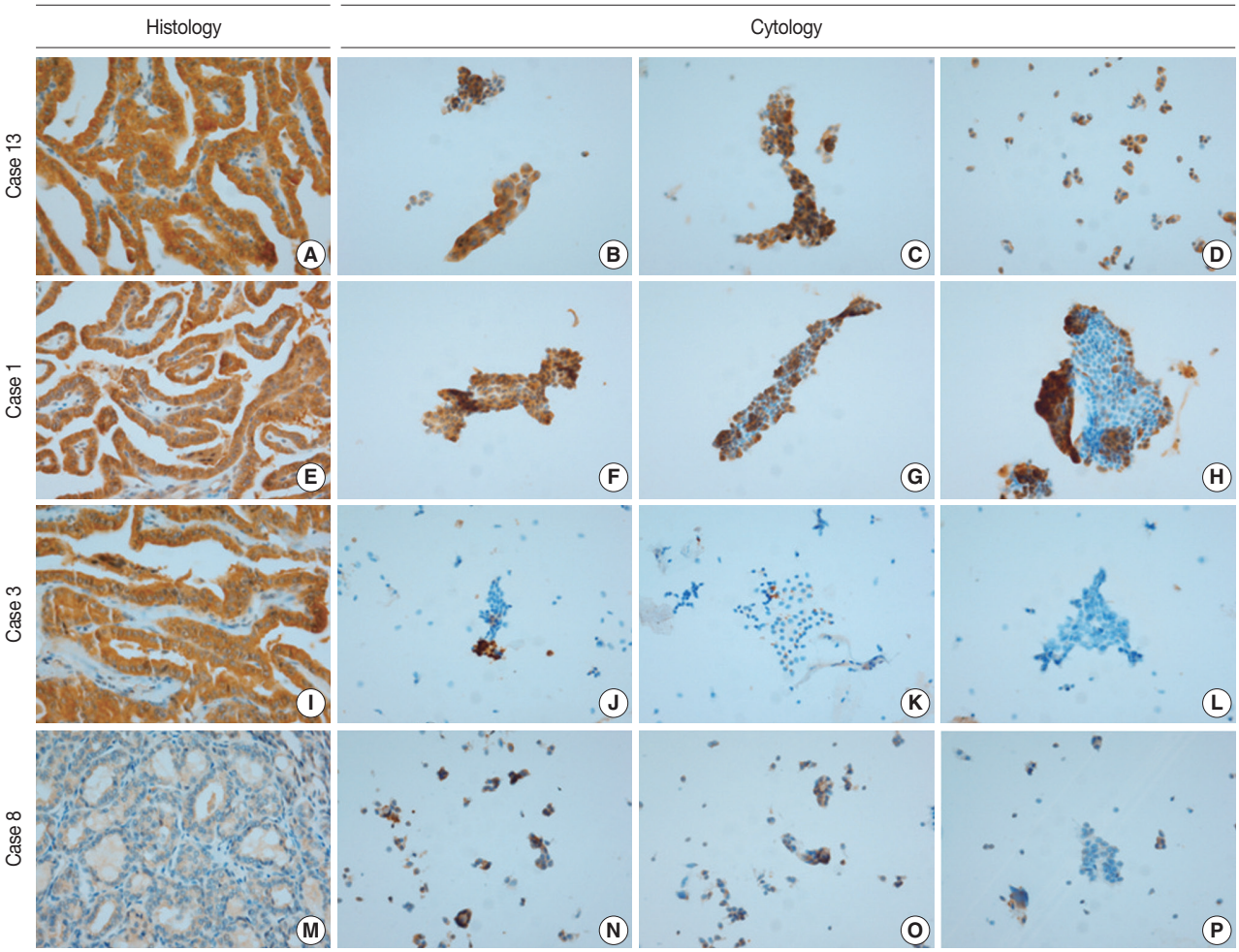


**Fig. 1.** The extent of VE1 immunoexpression in representative cases evaluated histologically (A, E, I, M) and cytologically (B–D, F–H, J–L, N–P). (A, E, I) Diffuse VE1 positivity in classic papillary thyroid carcinomas. (M) VE1 negativity in follicular variant papillary thyroid carcinoma. (B–D) Diffuse VE1 positivity in corresponding cytology. (F–H) Multifocal VE1 positivity in corresponding cytology. (J–L) Focal VE1 positivity in corresponding cytology. (N–P) Multifocal VE1 positivity in corresponding cytology.

ly resected *ex vivo* PTC specimens, because previous studies have suggested that the lower sensitivity and specificity of VE1 ICC compared to those of VE1 IHC might be related to the limitations of thyroid FNA cytology such as the extent of cellularity and the representative nature of the obtained thyroid tissue.[13,15,16] In the present study, all 21 LBC samples contained predominantly tumor cells, representing the cancerous area of each PTC specimen, and had an optimal cellularity for evaluation with VE1 ICC. Our data showed that the VE1 antibody had a higher sensitivity (94.7%) than that afforded by FNA cytology. Rossi *et al*.[15] and Lee *et al*.[13] reported sensitivities of 82.0% and 88.8%, respectively, when the VE1 antibody was evaluated in LBC samples. Wobker *et al*.[14] evaluated the VE1 antibody using smears of thyroid FNA material, but the detection sensitivity (63.6%) was less than that afforded by LBC samples.

Although the cytology specimen in the present study was more representative of the corresponding histology than the cytology of FNA samples, the VE1 immunostaining patterns in ICC differed from those in IHC. All PTCs with *BRAF*V600E mutation showed diffuse positivity in VE1 IHC, as in previous studies,[10,12,17] suggesting that the *BRAF*V600E mutation represents a clonal event during PTC development.[17] Using ICC, however, only 11 PTCs (57.9%) with the *BRAF*V600E mutation revealed diffuse positivity; other cases exhibited focal or multifocal positivity. Variations in the intensities and proportions of VE1-positive tumor cells in the same samples were also noted in earlier studies using FNA material.[14-16] Staining variability can be influenced by storage duration, technical problems, or fixation type.[14,15,18] In the present study, ICC on LBC was performed within 48 hours after sampling. In an attempt to eliminate technical problems, VE1 ICC was performed using different methods, but the results were similar (Appendix 1). It has been suggested that ethanol-based fixation destabilizes proteins not only in histology,[19] but also in cytology.[18] We used a methanol- and isopropanol-based preservative (CytoRich Red) containing 1% formalin as a fixative, which is known to be more compatible with ICC than an ethanol-based fixative.[18] Nonetheless, we found that VE1 ICC was less sensitive than IHC in detecting *BRAF*V600E mutation. Previous studies found that the extent of disagreement between ICC and IHC was 7.2%–34.7% and suggested that differences in fixation methods might explain the observed discrepancies.[14-16] Our results also indicate that differences in fixation between ICC and IHC are a major contributing factor resulting in different VE1 immunoexpression in the same tissue samples.

Upon IHC, VE1 was detected with high specificity, but the ICC specificity was 0% because one PTC harboring the *BRAF*V600E mutation was negative for VE1, while two follicular variant PTCs lacking the *BRAF*V600E mutation were positive for VE1 immunostaining. We varied the molecular and immunohistochemical methods used, but the results were similar (Appendices 2, 3). To evaluate the specificity, the number of wild-type PTC samples in the present study was too small. Nonetheless, false positivity of VE1 in thyroid cytology should not be underestimated. Nonspecific staining of colloids, macrophages, and follicles containing colloids or stroma has been suggested to hamper the interpretation of VE1 ICC.[13-16] One recent study showed that the VE1 antibody cross-reacted with certain ciliary structural proteins, inducing VE1 false positivity.[20] Some proteins expressed in endocrine organs, including α-ketoglutarate-dependent dioxygenase alk-B homolog 7, eukaryotic translation initiation factor 2-α kinase 4, polo-like kinase-1δ, potassium channel tetramerization domain-containing 4, and solute carrier family 4 (anion exchanger) member 3, also share sequence similarities with the peptide immunogen used to generate the VE1 antibody.[20] Such cross-reactivities might possibly explain the nonspecific staining of, or false-positivity for, VE1 in thyroid PTC samples.

This study has several limitations, mostly stemming from its small number of cases. Nonetheless, the results from the present study suggest that VE1 ICC is less specific than VE1 IHC in detecting the *BRAF*V600E mutation. For clinical application of the VE1 antibody in thyroid cytology, further evaluation and optimization of VE1 immunostaining in cytology specimens are essential.

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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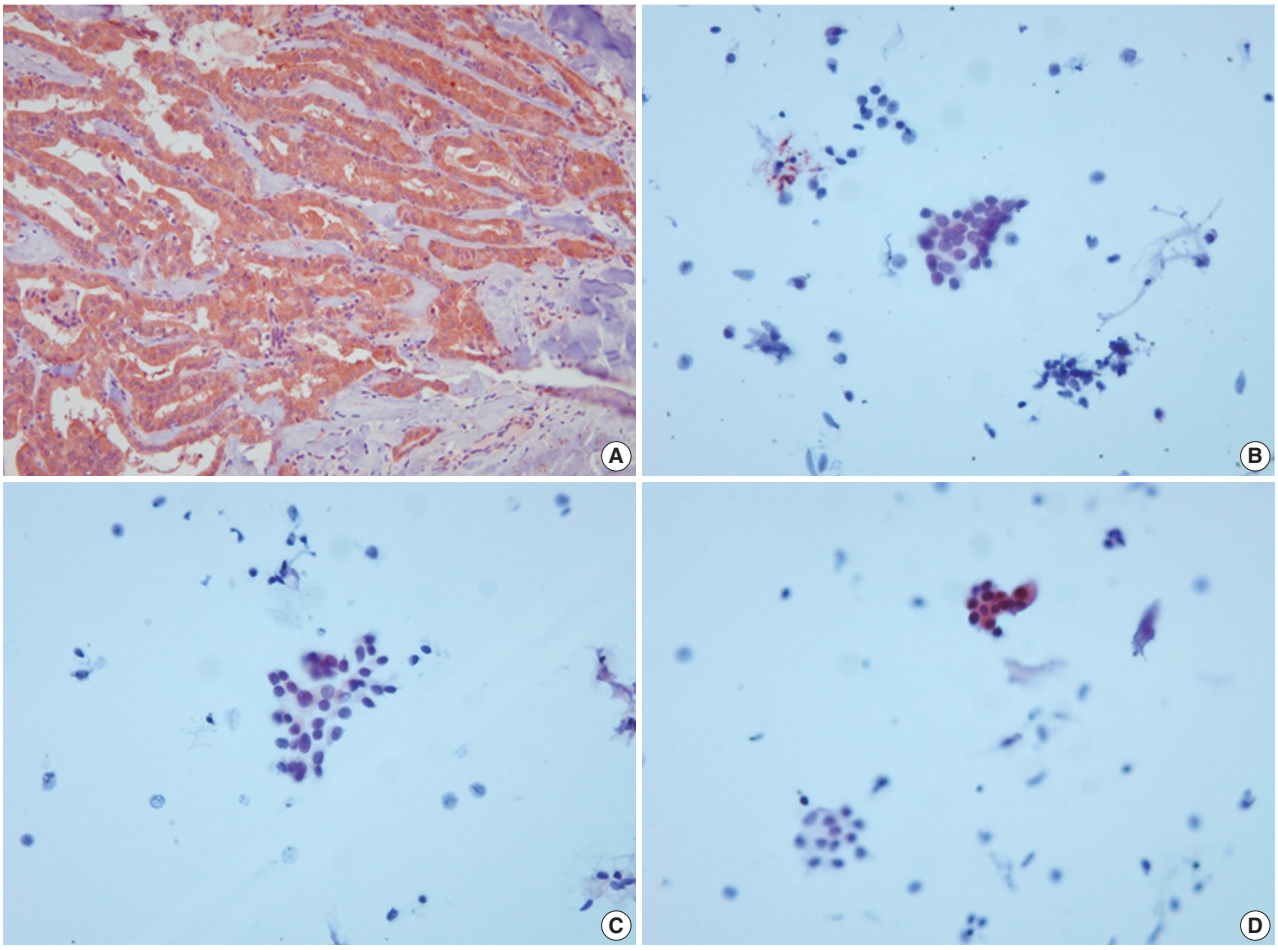


**Appendix 1.** VE1 immunoexpression in histology and cytology of case 3 with manual method using the Ultravision LP Detection System (Thermo Fisher Scientific). (A) Diffuse and strong VE1 expression in histology of case 3. (B–D) Focal VE1 expression in corresponding cytology.


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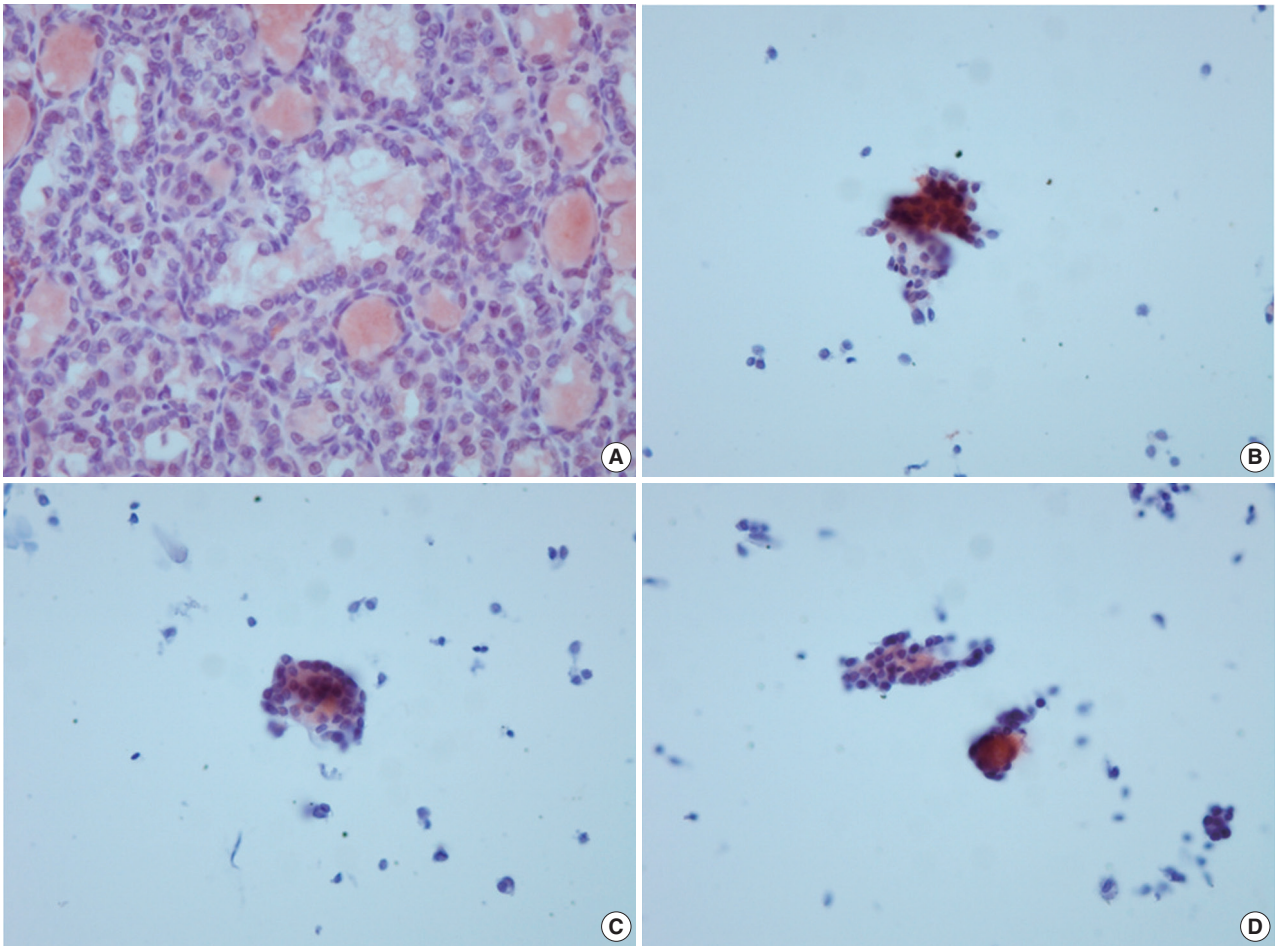
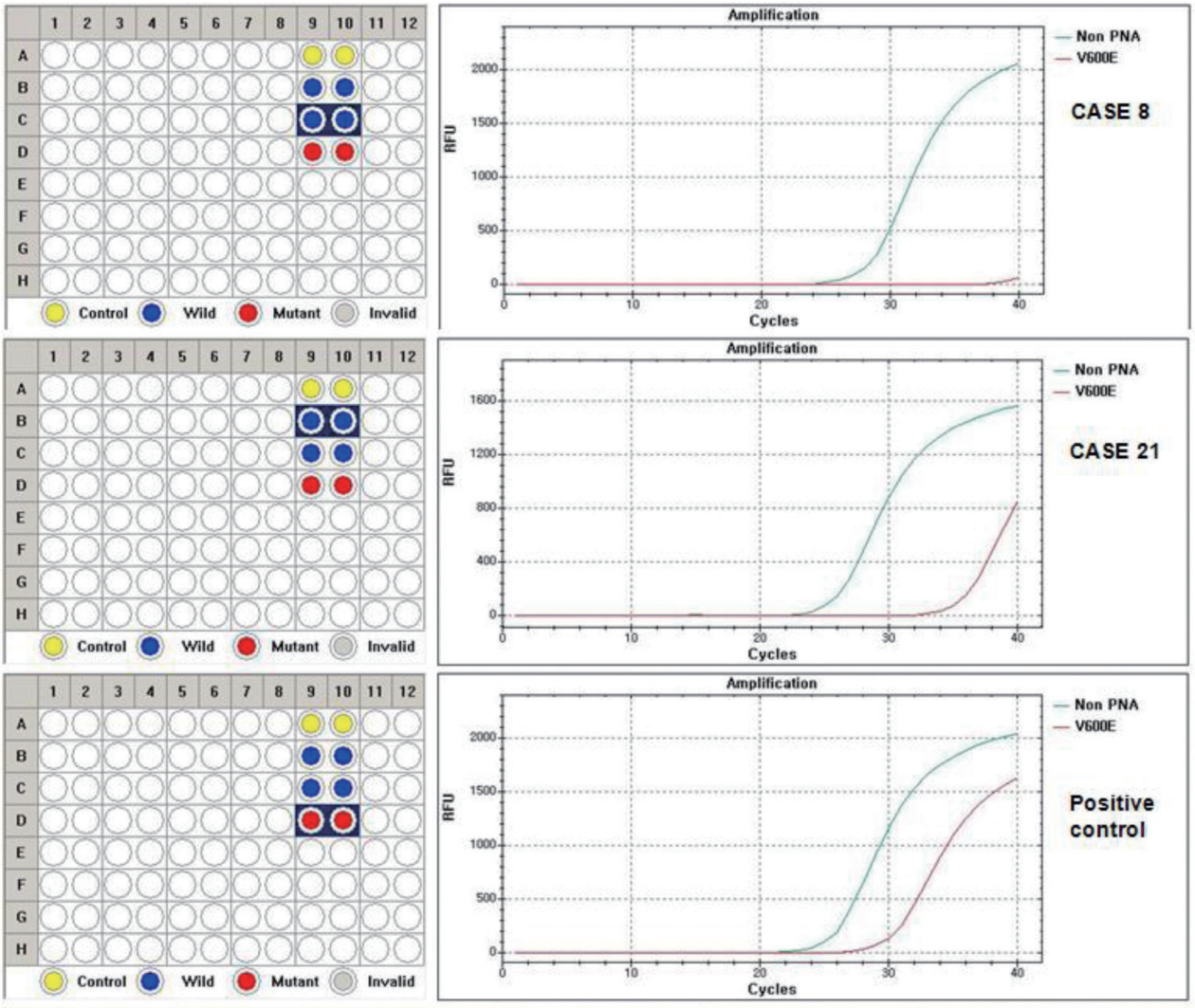



**Appendix 2.** VE1 immunoexpression in histology and cytology of case 8 with manual method using the Ultravision LP Detection System (Thermo Fisher Scientific). (A) Negative VE1 expression in histology of case 8. (B–D) Multifocal VE1 expression in corresponding cytology.




| Sample# | ID | Sample Information | | | Cycle Threshold(Ct) | | ΔCt | | Result |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Non-PNA | PNA | ΔCt-2 | ΔCt-1 | |
| Control | | | | | 24.90 | 35.74 | | | |
| 1 | CASE 8 | | | | 25,31 | 35,28 | 9,97 | -0,28 | Wild |
| 2 | CASE 21 | | | | 27,25 | 38,00 | 10,75 | -3,00 | Wild |
| 3 | Positive Con | | | | 24,98 | 29,37 | 4,40 | 5,63 | BRAF V600E |

**Appendix 3.** No *BRAF*V600E mutation in case 8 and case 21 by peptide nucleic acid clamping method.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■ CASE STUDY ■

# IgG4-Related Disease Presented as a Mural Mass in the Stomach

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Isolated gastric IgG4-related disease (IgG4-RD) is a very rare tumefactive inflammatory condition, with only a few cases reported to date. A 48-year-old woman was incidentally found to have a subepithelial tumor in the stomach. Given a presumptive diagnosis of gastrointestinal stromal tumor or neuroendocrine tumor, she underwent wedge resection. The lesion was vaguely nodular and mainly involved the submucosa and proper muscle layer. Microscopically, all classical features of type I autoimmune pancreatitis including lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, and numerous IgG4-positive plasma cells were seen. She had no evidence of IgG4-RD in other organs. Although very rare, IgG4-RD should be considered one of the differential diagnoses in the setting of gastric wall thickening or subepithelial mass-like lesion. Deep biopsy with awareness of this entity might avoid unnecessary surgical intervention.

**Key Words:** Immunoglobulin G4; Stomach; Autoimmune diseases; Granuloma, plasma cell

IgG4-related disease (IgG4-RD) is a recently recognized inflammatory condition characterized by several clinico-pathologic features: a tendency to form mass-like lesions, dense lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, increased IgG4-expressing plasma cells, and often but not always elevated serum IgG4 level.[1-4] IgG4-RD can affect multiple organs simultaneously or can present as a solitary, mass-like lesion. When IgG4-RD presents as a solitary, mass-like lesion, it might be misinterpreted clinically or radiologically as a neoplasm, resulting in overtreatment. IgG4-RD usually affects the pancreas, biliary tree, liver, salivary glands, lacrimal glands, and retroperitoneum; however, involvement of the gastrointestinal (GI) tract is very rare, and diagnostic criteria have not yet been well established. A recent review proposed two types of IgG4-RD of the GI tract. One is diffuse wall thickening, and the other is polyp or mass-like lesion.[5] To date, there have been eight IgG4-RDs reported in the stomach regardless of the presence in other organs. Here, we describe the ninth case of IgG4-RD in the stomach, which presented as an isolated mass-like lesion without involvement of any other organ.

## CASE REPORT

A 48-year-old, previously healthy woman was found to have a subepithelial tumor during health screening endoscopy (Fig. 1A). Abdominal computed tomography demonstrated a 3.6×2.2 cm, well-defined, solid, enhancing submucosal mass on the posterior wall of the stomach midbody (Fig. 1B). Radiologic differential diagnoses included GI stromal tumor and neuroendocrine tumor. No remarkable findings were observed in other organs. Seven years ago, she had undergone modified radical mastectomy for breast cancer. There was no further history, symptoms, or signs of systemic disease, and laboratory tests were unremarkable. Serum IgG4 level was not measured preoperatively. Given a presumptive diagnosis of submucosal neoplasm, wedge resection was performed.

Grossly, the lesion was a poorly circumscribed, yellowish grey, fusiform mass involving the area from the submucosa to subserosa (Fig. 2A). The overlying mucosa was intact, and there was no ulceration. Microscopically, the mass showed marked fibrosis, often in a storiform pattern of many lymphoid follicles with well-formed germinal centers, and diffuse inflammatory cell infiltration. The infiltrated inflammatory cells were mainly lymphocytes and plasma cells, but some eosinophils were also found

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(Fig. 2B–D). Obliterative phlebitis was occasionally observed in elastic staining (Fig. 2E). There were numerous IgG4-positive cells throughout the lesion, and the number of IgG4-positive plasma cells was up to 210 per high-power field (Fig. 2F). The IgG4 to IgG-positive cell number ratio was about 85%; however, there were only a few IgG4-positive cells in the mucosa. There was no significant myofibroblastic proliferation or immunostaining for anaplastic lymphoma kinase; therefore, the possibility of inflammatory myofibroblastic tumor was excluded. We concluded that this lesion was IgG4-RD. The patient's postoperative course was uneventful, and she was discharged without any complications. No recurrence was observed during the 10-month follow-up period.

## DISCUSSION

Here, we described a case of isolated gastric IgG4-RD presenting as a fusiform mural mass mimicking neoplasm, such as GI stromal tumor or neuroendocrine tumor. To the best of our knowledge, this is the ninth case of gastric IgG4-RD. Histologically, this case demonstrated all the important features of IgG4-RD, including dense lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, and abundant IgG4-positive cells. Although other diagnostic criteria, such as elevated serum IgG4 level or response to steroid therapy, could not be confirmed due to the clinical presentation, typical histopathologic features led us to consider IgG4-RD.

Increased IgG4-positive plasma cells can be seen in other organs and in many conditions, including non-specific chronic inflammation, lymphoma, and other malignancies.[4,6-8] However, these lesions lack other characteristic histopathologic findings, such as storiform fibrosis and oblierative phlebitis, as has been described in the consensus statement on the pathology of IgG4-RD.[4] Although abundant IgG4-positive plasma cell infiltration

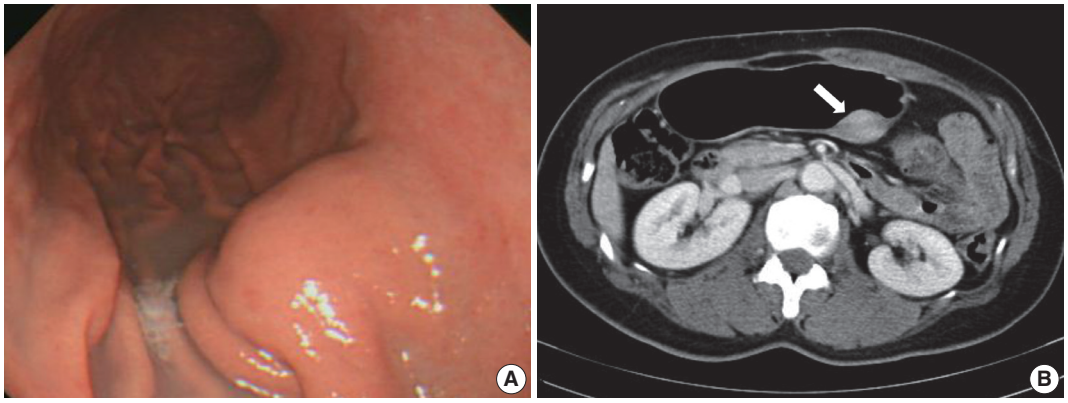


**Fig. 1.** Endoscopic and abdominal computed tomography scan images. (A) Localized smooth elevation of the gastric mucosa without mucosal fold abnormality. (B) A well-defined, solid, enhancing mass measuring 3.6×2.2 cm at the posterior wall of the stomach midbody (arrow).

**Table 1.** Clinicopathologic features of the cases of probable IgG4-related disease in the stomach

| Reference | Sex/Age (yr) | Endoscopic finding | Sites | Invovement | Serum IgG4 | Procedure | Associated condition |
|---|---|---|---|---|---|---|---|
| Baez *et al.*[10] | M/58 | Nodule, 1.4 cm | Fundus and body | Mucosa | Normal | Steroid | AIP, IgG4-related sialadenitis |
| Kaji *et al.*[11] | M/74 | Mutiple polyps with erosion and redness | Body | Mucosa | Increased | NA | AIP |
| Chetty *et al.*[12] | F/45 | Nodule, 1.5 cm | Fundus | Submucosa | Normal | WR | Raynaud's disease |
| Chetty *et al.*[12] | M/60 | Multiple nodules, up to 2.2 cm | Antrum | Proper muscle to submucosa | NA | DG | Autoimmune polyendocrinopathy |
| Rollins *et al.*[13] | F/75 | Polypoid lesion, 5.6 cm | Body | Submucosa | NA | WR | None |
| Na *et al.*[9] | M/56 | Nodule, 0.8 cm | Low body | Submucosa | NA | ESD | Type 2 diabetes mellitus |
| Kim *et al.*[14] | F/59 | Mass, 3.3 cm | Midbody | Proper muscle | Normal | WR | None |
| Kim *et al.*[14] | F/54 | Mass, 2.1 cm | NA | Proper muscle to submucosa | Normal | WR | None |
| Present case | F/48 | Mass, 3.6 cm | Midbody | Submucosa to subserosa | NA | WR | None |

M, male; AIP, autoimmune pancreatitis; NA, not available; F, female; WR, wedge resection; DG, distal gastrectomy; ESD, endoscopic submucosal dissection.


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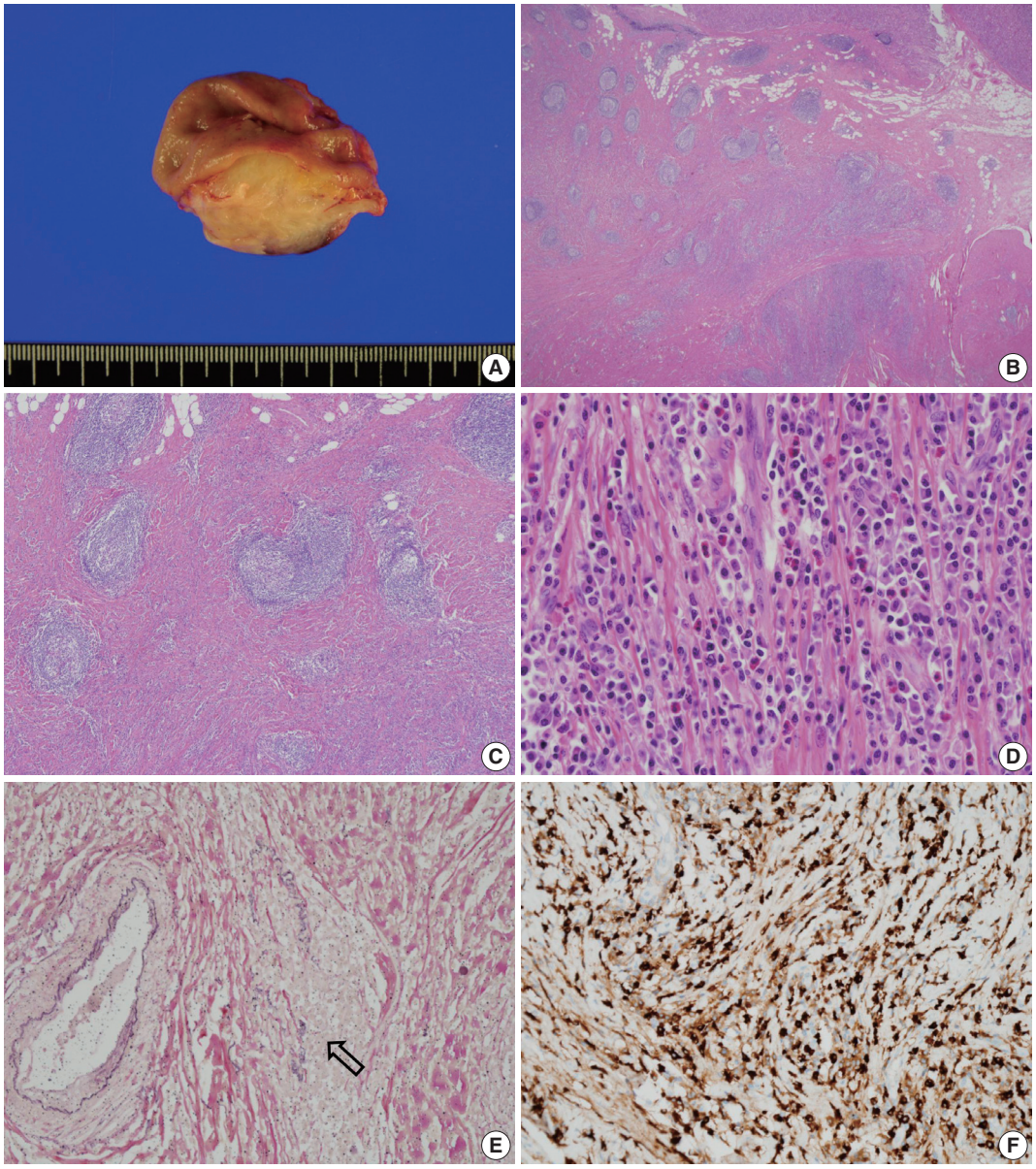


**Fig. 2.** Gross and microscopic appearance of the resected specimen. (A) An ill-defined, yellowish grey mass involves the full thickness of the gastric wall except the mucosa. (B) The mass is not encapsulated and is filled with fibrotic tissue and multiple lymphoid follicles. (C) Storiform fibrosis is observed between lymphoid follicles. (D) Numerous plasma cells and many eosinophils are noted in the fibrotic stroma. (E) Obliterative phlebitis is demonstrated in elastic staining (arrow). Note the residual elastic fiber of the obliterated vein (Van Gieson). (F) Numerous IgG4-positive cells are noted in the sclerotic area.

is not uncommon in the GI tract in the setting of autoimmune pancreatitis, the simple presence of IgG4-positive cells does not justify a diagnosis of IgG4-RD in the absence of other gross and microscopic features, such as tumefactive nature, storiform fibrosis, and obliterative phlebitis.

Including the present case, there have been nine cases of mass-forming IgG4-RD in the stomach.[9-14] A case of probable IgG4-RD that presented as a gastric ulcer has also been reported,[15,16] but we excluded this case from the present review. As is summarized in Table 1, most gastric IgG4-RD was detected in middle age (mean, 58.8 years; range, 45 to 75 years), and men and women were affected equally, although the total number of patients is likely too small to reveal any meaningful data. Seven patients had solitary nodules or masses, whereas two patients had multiple polyps or nodules. The two patients with multiple lesions also had autoimmune pancreatitis and autoimmune polyendocrinopathy, respectively. Four of the seven cases showing a solitary lesion had no sign of multi-organ involvement. Most cases

of gastric IgG4-RD (six of nine) involved the submucosal layer of the gastric body. Proper muscle or mucosa was variably involved. Serum IgG4 was increased only in patients with associated autoimmune pancreatitis. Most gastric IgG4-RD patients were treated surgically except for one patient with autoimmune pancreatitis who was treated with steroid.

Steroid treatment is the first therapeutic option in IgG4-RD,[17] but all reported isolated gastric IgG4-RD cases were surgically resected, presumably because they are rare and difficult to diagnose without pathologic examination of a resected specimen. Unnecessary surgery might be avoided if the possibility of IgG4-RD is kept in mind and careful pathologic evaluation including IgG4 immunostaining is performed on a deep biopsy obtained using endoscopic ultrasonography.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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# Gastric-Type Extremely Well-Differentiated Adenocarcinoma of the Stomach: A Challenge for Preoperative Diagnosis

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Gastric-type extremely well-differentiated adenocarcinoma (EWDA) is a rare type of gastric adenocarcinoma characterized by infiltration of well-formed mucinous glands with little or no nuclear atypia, which resemble foveolar epithelium or pyloric glands. Because of its high degree of differentiation, preoperative biopsy diagnosis of gastric-type EWDA is very difficult. We encountered a case of gastric-type EWDA, manifesting as a Borrmann type 4 lesion, in a 47-year-old man. Despite four repeated biopsies, the preoperative biopsy diagnosis was not conclusive due to the scarcity of diagnostic tumor cells and lack of knowledge regarding the unusual histologic findings of gastric-type EWDA. We herein describe the histologic findings of gastric-type EWDA in detail, with the aim of facilitating a preoperative biopsy diagnosis and understanding of this rare type of gastric adenocarcinoma.

**Key Words:** Stomach neoplasms; Extremely well-differentiated adenocarcinoma; Gastric-type

Extremely well-differentiated adenocarcinoma (EWDA) is a rare type of gastric adenocarcinoma, which accounts for less than 0.2% of all gastric cancers.[1] EWDA, as its name suggests, is highly differentiated and almost indistinguishable from regenerating atypia or inflammatory changes. Such a high degree of differentiation and minimal cellular atypia commonly lead to diagnostic difficulties, particularly with preoperative forceps biopsy.[1,2] Based on histological and immunohistochemical differences, EWDAs can be classified into two variants: intestinal-type and gastric-type.[2] In 1999, Endoh *et al*.[3] first coined the term "extremely well differentiated adenocarcinoma of the stomach" to describe well-differentiated adenocarcinoma with low-grade nuclear atypia and morphology mimicking intestinal metaplasia, which is currently considered a prototype of intestinal-type EWDA. Meanwhile, gastric-type EWDAs are quite different from intestinal-type EWDAs, which consist of benign-looking mucous cells resembling foveolar epithelium, mucus neck cells, or pyloric glands and contain gastric phenotype mucin.[2,4,5] The frequency of diagnosing intestinal-type EWDA with biopsy specimens prior to resection is increasing, since characteristic architectural features of the neoplastic glands, which resemble the shape of the letter W, H, Y, or X, have been reported to be the most informative and useful diagnosis criteria.[6] However, since little is known about gastric-type EWDA due to its rarity, its preoperative diagnosis with biopsy remains challenging. The present case emphasizes the importance of being aware of this rare type of adenocarcinoma in order to make a preoperative biopsy diagnosis.

## CASE REPORT

A 47-year-old man presented with epigastric discomfort and dyspepsia. Esophagogastroduodenoscopy revealed a diffusely infiltrative submucosal lesion involving the upper body of the stomach along the greater curvature (Fig. 1A). The overlying mucosa was intact and non-ulcerative, but it showed tiny scattered mucosal openings with extruding mucus (Fig. 1B). Abdominal computed tomography scan demonstrated diffuse wall thickening with suspicious perigastric infiltration, consistent with Borrmann type 4 gastric cancer. The patient underwent four consecutive gastrofiberscopic examinations, with biopsies consisting of more than 10 pieces, but all biopsies were negative for adenocarcinoma. Subsequently, the patient underwent a diagnostic laparotomy and total gastrectomy and was diagnosed with gastric-type EWDA (pT4aN3aM1, stage IV). Microscopic examination revealed numerous irregular-shaped mucinous

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glands that diffusely extended from the submucosal layer to the visceral peritoneum (Fig. 1C). The mucosal layer was spared for the most part, with only some carcinoma glands found in the deep portion of the mucosa. The tumor glands consisted of mucin-rich columnar cells with basally located, small, bland-looking nuclei mimicking hyperplastic foveolar epithelium or dilated pyloric glands (Fig. 1D), which deeply infiltrated the proper muscle with no desmoplastic stromal reaction (Fig. 1E). There were also areas showing distinctly lobular proliferation of small glands often surrounding a larger central duct (Fig. 1F); such areas were frequently observed in the submucosa, and the central dilated duct occasionally opened through the overlying mucosal surface (Fig. 1G). The tumor cells were diffusely positive for MUC5AC (Fig. 1H), human gastric mucin (HGM), and carcinoembryonic antigen (CEA); focally positive for MUC6; and negative for MUC2, CD10, and p53. The Ki-67 labeling index was low (5%). We retrospectively reviewed the preoperative biopsy specimens and found a few scattered carcinoma glands in the basal portion of the oxyntic-type corpus mucosa (Fig. 2A). Despite not being atypical enough to be considered definite carcinoma, the glands were indisputably noticeable, with large size, abundant clear cytoplasm, mild nuclear atypia, and unusual

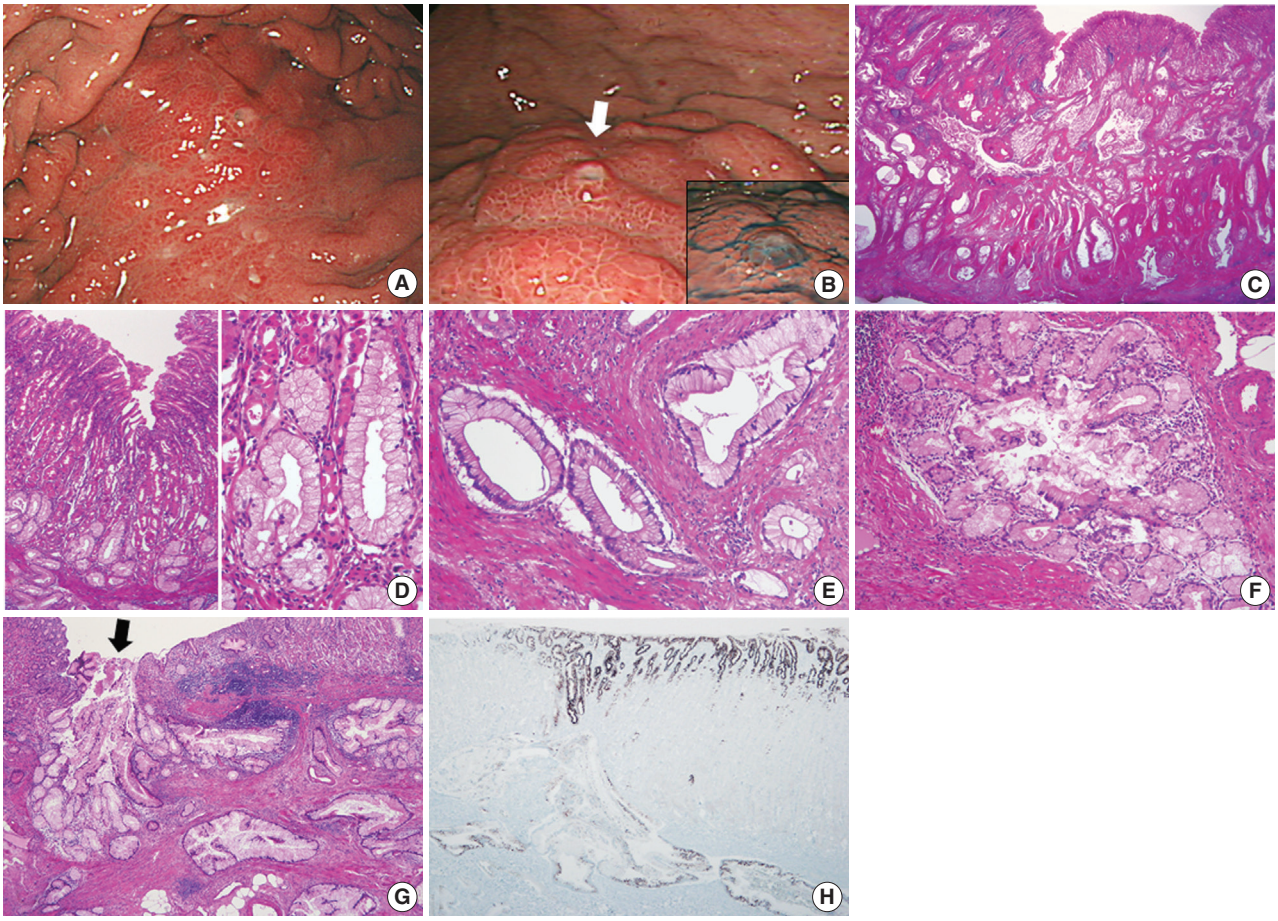


**Fig. 1.** (A, B) Esophagogastroduodenoscopy. (A) A submucosal infiltrative lesion is observed in the upper body. (B) The overlying mucosa is mainly intact with scattered small openings with protruding mucus (arrow and inset). (C–E) Representative microphotographs of invasive carcinoma. (C) Low-magnification view demonstrates that the entire gastric wall is infiltrated by irregular neoplastic glands showing frequent cystic dilatation and intraluminal mucinous material. (D) The mucosal surface is mainly intact, and the neoplastic glands partly involve the deep portion of the mucosa. The neoplastic glands with basally-located, small, bland nuclei and abundant mucin-containing cytoplasm mimic normal gastric foveolar epithelium or pyloric glands. Compared to the adjacent normal glands, they are slightly larger in size and more irregular in shape. (E) Infiltrating neoplastic glands show no significant cytological or architectural atypia and no desmoplastic stromal reaction. (F, G) Representative microphotographs of lobular endocervical glandular hyperplasia-like areas. (F) A cluster of small glands surrounds a central large duct in a distinct lobular arrangement with a sharp border. (G) Such areas are mostly observed in the submucosal layer, and occasionally a central dilated duct with mucinous materials opens through the overlying mucosa (arrow), which is in concordance with mucus protrusion on endoscopy (arrow in Fig. 1B). (H) Immunostaining for MUC5AC is diffusely positive in the tumor glands and foveolar epithelial cells.


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location compared to normal mucous glands (Fig. 2B, C). Those atypical glands were more distinguishable on immunostaining for HGM, CEA, and MUC5AC (Fig. 2D).

## DISCUSSION

To date, only nine cases (including the present one) of gastric-type EWDA have been reported in the English literature. The clinical and pathological findings of these patients are summarized in Table 1. The patients were all male with ages ranging from 47 to 81 years. The tumors in all reported cases were located in the upper or middle third of the stomach. Grossly, they were polypoid masses or submucosal tumors mimicking Borrmann type 4 gastric cancer. Preoperative biopsy findings were available in seven cases, five of which were diagnosed as benign lesions, one as a suspicious carcinoma, and one as definite carcinoma on repeat biopsy. It is very difficult to diagnose gastric-type EWDA in a preoperative biopsy specimen, especially in

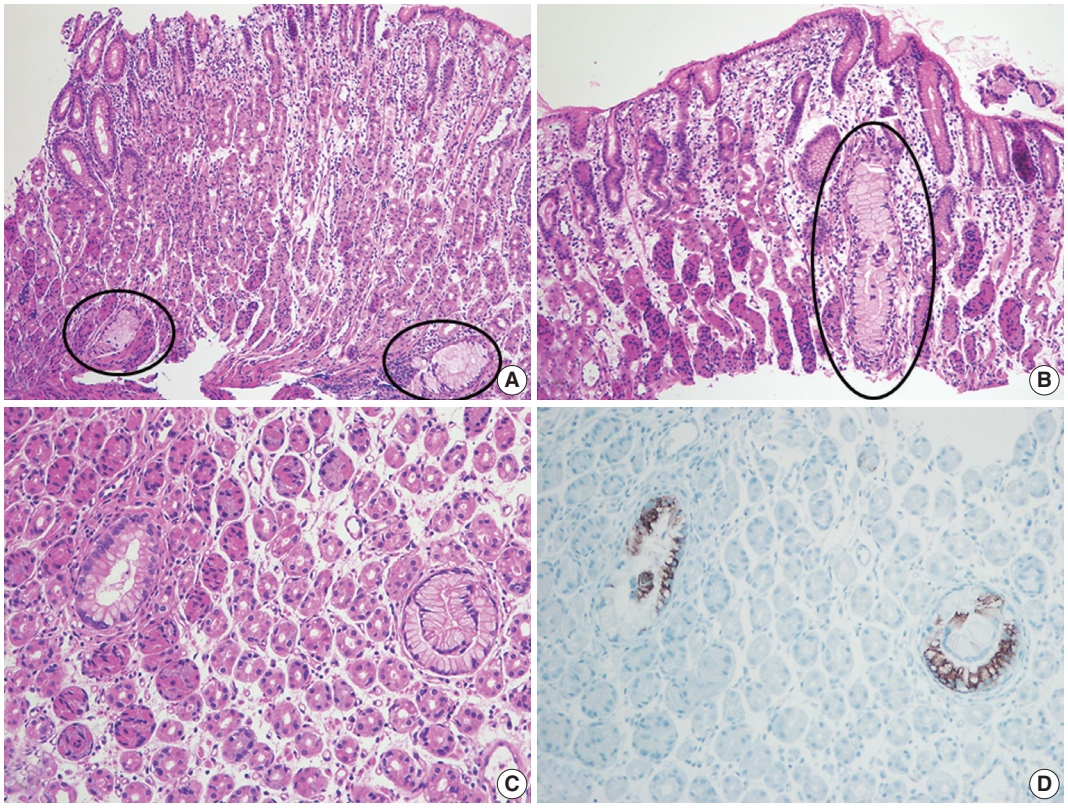


**Fig. 2.** Preoperative biopsy findings. (A, B) There are a few atypical mucinous glands (circles) that are noticeably large in size and observe frequently in the basal portion of the corpus mucosa. (C) Such mucinous glands display mild nuclear atypia, but they are not atypical enough to be recognized as adenocarcinoma. (D) These glands are highlighted by MUC5AC immunostaining.

**Table 1.** Clinical and pathological findings of previously published cases of gastric-type EWDA in the English literature

| Case No. | Reference | Sex/Age (yr) | Location | Size of tumor (cm) | Macroscopic type | Depth of invasion | Preoperative biopsy diagnosis |
|---|---|---|---|---|---|---|---|
| 1 | Niimi *et al.*[1] | M/58 | Upper third | 7 | Borrmann type 4 | T3 | Benign |
| 2 | Yao *et al.*[2] | M/81 | Middle third | 5.5 | EGC type 0-I | T1b | Benign |
| 3 | Yao *et al.*[2] | M/51 | Upper third | 2.5 | EGC type 0-IIa | T1b | NA |
| 4 | Yao *et al.*[2] | M/63 | Middle third | 8 | Borrmann type 1 | T3 | Benign |
| 5 | Yao *et al.*[2] | M/76 | Upper third | 3.5 | Borrmann type 1 | T3 | Suspicious for CA |
| 6 | Yao *et al.*[2] | M/57 | Upper third | 5 | Borrmann type 1 | T3 | NA |
| 7 | Nokubi *et al.*[4] | M/60 | Cardia | NS | Borrmann type 4 | T4b | CA on repeated biopsy |
| 8 | Lee[5] | M/67 | Cardia | 7 | Borrmann type 1 | T3 | Benign |
| 9 | Present case | M/47 | Upper third | 6.5 | Borrmann type 4 | T4a | Benign |

EWDA, extremely well-differentiated adenocarcinoma; M, male; EGC, early gastric cancer; NA, not assessed; CA, carcinoma; NS, not stated.

patients with Borrmann type 4 lesions. Nevertheless, the most important aspect in diagnosing gastric-type EWDA in a biopsy specimen is observation of the subtle atypical changes of mucous glands: mucous glands with conspicuously abundant mucinous cytoplasm, mild nuclear atypia, and slightly larger size should raise suspicion of gastric-type EWDA, particularly when they are abnormally clustered or seen in the deep portion of the corpus mucosa that is usually devoid of well-formed mucous glands. Additional immunostaining for CEA and gastric phenotype mucin can help to confirm the diagnosis.

The histologic characteristics of gastric-type EWDA, including deep invasion of extremely well-formed mucinous glands, minimal cellular atypia, and no significant desmoplastic stromal reaction, resemble those of minimal deviation adenocarcinoma (MDA) of the uterine cervix. Therefore, gastric-type EWDA has been considered as the gastric counterpart of MDA.[4,5] According to the new World Health Organization classification published in 2014, MDA is classified as an extremely well-differentiated form of gastric-type endocervical mucinous adenocarcinoma.[7] Lobular endocervical glandular hyperplasia (LEGH) has been described as a distinctive hyperplastic lesion of the endocervix characterized by lobular proliferation of small, rounded, non-invasive glands often surrounding a central dilated gland. It has been postulated that LEGH is a precursor lesion of MDA.[8,9] In the present case, we found LEGH-like areas with distinct histological characteristics of LEGH that occurred concurrently with invasive mucinous carcinoma. In addition, they were predominantly observed in the submucosal layer, just as LEGH is usually confined to the inner half of the endocervical wall. LEGH-like lesions have never been described in the stomach before, which may provide evidence to support gastric-type EWDA being the gastric counterpart of MDA of the uterine cervix.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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■ BRIEF CASE REPORT ■

# Double Para-testicular Cellular Angiofibroma and Synchronous Testicular Microlithiasis

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Cellular angiofibroma is a rare benign mesenchymal tumor. Since first described by Nucci *et al.* in 1997,[1] this distinct histopathologic entity has been reported in a series of studies[2-4] and case reports.[5-7] The tumor is characterized by two principal components, cellular spindle cells and prominent blood vessels.[1] Mild cytologic atypia and a few mitotic figures have been demonstrated in some cases.[2,4] The tumor occurs mainly in the inguinoscrotal region in men.[2-5,7] The molecular pathogenesis of cellular angiofibroma is largely unknown, although retinoblastoma 1 (RB1) and forkhead box protein O1 (FOXO1) have been implicated in some cases.[3,7] Testicular microlithiasis is a relatively rare condition characterized by calcifications within the seminiferous tubules. Two different types of testicular microlithiasis, hematoxylin bodies and laminated calcifications, have been described.[8] Many laminated calcifications have been associated with atrophic seminiferous tubules or germ cell neoplasia.[8,9] Here, we present a case of double para-testicular cellular angiofibroma and synchronous testicular microlithiasis.

## CASE REPORT

The present study was approved by the Institutional Review Board of Kangwon National University Hospital (KNUH-2015-05-011). A 40-year-old man with two children presented with complaints of self-limited hemospermia for one month. There was no history of exposure to sexually transmitted diseases or penile trauma. He had no previous episodes of hematuria. A solid mass-like lesion was first discovered in the left scrotum by self-examination. Physical examination revealed a non-tender, firm, slightly mobile, and well-demarcated scrotal mass. Ultrasound examination revealed an approximately 1.2-cm solid mass in the tail of the epididymis (Fig. 1A). The mass showed an isoechoic and mild hypervascular pattern. However, the left testis revealed no mass lesion. Prostate examination, urinalysis, and other laboratory studies were unremarkable. The patient underwent a left simple orchiectomy. On gross examination, two para-testicular masses were identified (Fig. 1B). One mass (1.2×0.8×0.7 cm) was present near the tail of epididymis. The other mass (1.1×0.6×0.5 cm) was located near the head of epididymis. The two masses were well-circumscribed, and 1.3 cm and 0.5 cm apart from the testis, respectively. The cut surface of the mass was grayish white and firm. Microscopically, the two masses showed histologically identical features, which were loosely arranged, bland spindle cells in a myxoid to collagenous stroma and prominent small- to medium-sized blood vessels (Fig. 2A). Two mitotic figures per 50 high power fields were identified only in the large mass (Fig. 2B). A few atypical spindle cells were also present (Fig. 2C). Immunohistochemically, tumor cells of the two masses were mostly positive for vimentin (1:200, Lab Vision, Fremont, CA, USA). Most perivascular spindle cells and some stromal spindle cells were positive for smooth muscle actin (Bond RTU Ab, alpha sm-1, Leica Biosystems, Newcastle upon Tyne, UK). Staining for CD34 (1:200, Lab Vision) was negative in the stromal spindle cells but positive in the perivascular spindle cells and endothelial cells. Tumor cells were negative for S-100 protein (1:100, Lab Vision), desmin (1:100, Zymed, San Francisco, CA, USA), and pan-cytokeratin (1:100, Leica). A moderate num-

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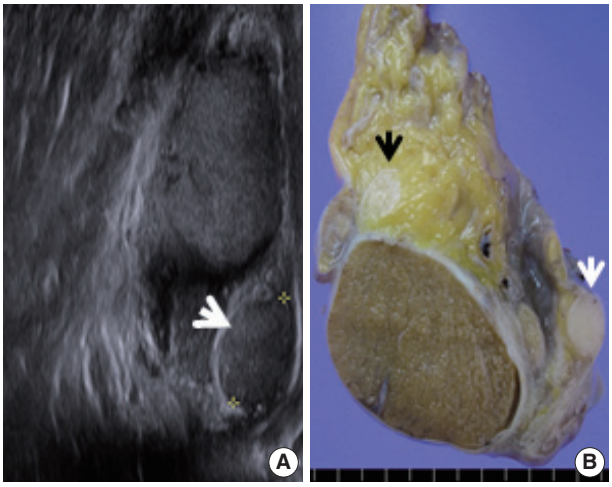

**Fig. 1.** Ultrasound examination and gross evaluation of orchiectomy specimen. (A) On ultrasound examination, there is an isoechoic mass in the tail area of the epididymis (white arrow). On the cut surface of the gross specimen, solid soft tissue is noted near the tail of the epididymis (A, white arrow) and near the head of the epididymis (B, black arrows).

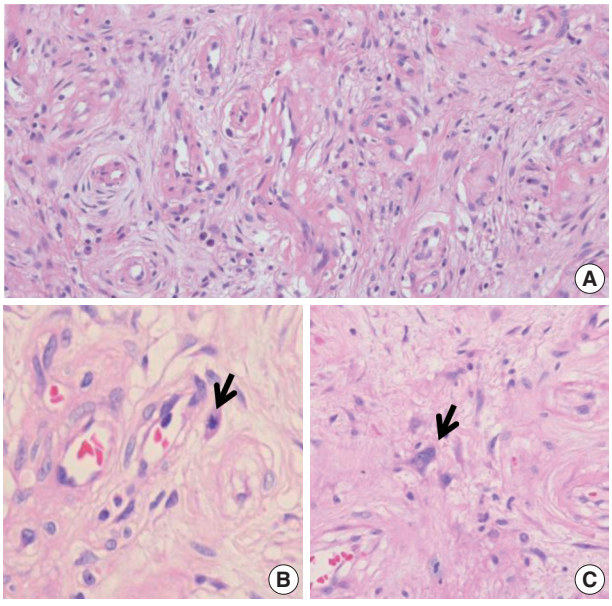

**Fig. 2.** Histopathologic features of the tumor. (A) The tumor is composed of spindle-shaped mesenchymal cells and thick-walled blood vessels. (B) Two mitotic figures per 50 high-power fields are identified in the mass. There is one of the mitotic cells (arrow). (C) A few stromal cells show atypical nuclear morphology.

ber of lymphocytes and plasma cells were scattered diffusely throughout the stroma of the tumor. Most of these lymphocytes were CD8+ cytotoxic T cells. The two masses were consistent with cellular angiofibroma. The testicular parenchyma and spermatic cord were grossly unremarkable. On microscopic examination of the testis, some atrophic seminiferous tubules were present and showed hyalinized degenerative change and contained corpora amylacea-like laminated eosinophilic bodies (Fig. 3). The remaining non-atrophic seminiferous tubules were unremarkable.

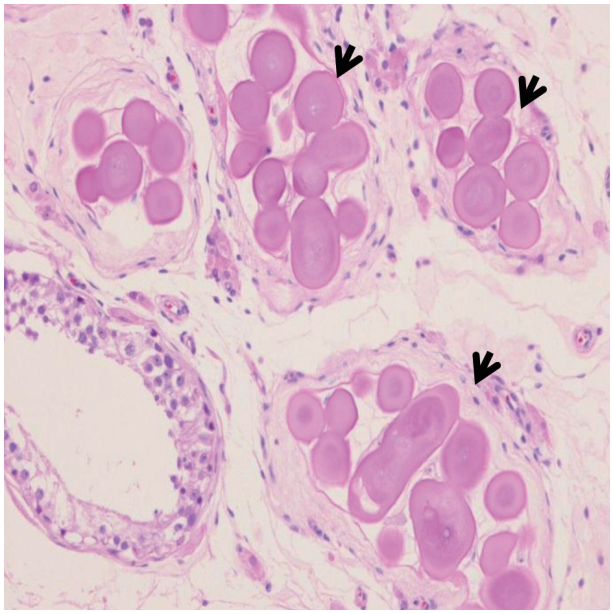

**Fig. 3.** Intratubular microcalcification. There are numerous completely hyalinized ghost tubules with corpus amylacea-like laminated intratubular bodies (arrows).

## DISCUSSION

Cellular angiofibroma usually occurs as a solitary and circumscribed lesion in the inguinoscrotal region of men.[1-5,7] Multiple cellular angiofibromas are very rare. To our knowledge, double cellular angiofibromas have not been reported.[1-7] Although the pathogenesis of multiple cellular angiofibroma is unknown, we speculate that it might be associated with certain cytogenetic alterations.[10] Cellular angiofibroma appears to be closely related to spindle cell lipoma and mammary-type myofibroblastoma, which show overlapping histologic and immunophenotypic features.[4] Recent evidence has shown that these three tumors have similar common molecular genetic features, such as monoallelic deletion of RB1 and FOXO1, both of which reside within 13q14.[3,7] One of the interesting findings of the testicular parenchyma in this report was the presence of corpora amylacea-like bodies, suggesting laminated testicular microlithiasis. Testicular microlithiasis is relatively rare, and the exact incidence in normal and diseased testes is unknown.[8,9] Laminated testicular microlithiasis has been associated with normal, cryptorchid testis, germ cell tumor, or atrophic seminiferous tubules.[8] In this case, laminated


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testicular microlithiasis was associated with atrophic seminiferous tubules. Although the patient presented with self-limited hemospermia, a direct relationship between microcalcification and hemospermia could not be determined. The patient has been followed up, and no recurrence has been reported. In summary, we described a rare para-testicular double cellular angiofibroma with synchronous lesion of testicular microlithiasis. Although these two lesions might represent a coincidental finding, the relationship between these two lesions awaits further elucidation.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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■ BRIEF CASE REPORT ■

# Indeterminate Dendritic Cell Tumor: A Case Report of a Rare Langerhans Cell Lineage Disease

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Indeterminate dendritic cell tumor (IDCT) is a very rare neoplastic disorder composed of so-called indeterminate cells.[1] Although it was first described in the early 1980s,[2] its pathogenesis and cellular origin have not been completely defined. These indeterminate cells morphologically and immunophenotypically resemble Langerhans cells, as they are immunopositive for S100 protein and CD1a. However, they typically lack both Birbeck granules and langerin expression.[1,3]

Due to its rarity and similarity to Langerhans cell lesions (LCL), IDCT can be misdiagnosed; however, the pathobiology of IDCT is different from that of LCL. Even though the clinical behavior of IDCT has not yet been firmly established, no direct mortality due to IDCT has been reported so far.[1,4] Recently, we experienced a case of IDCT with typical histologic features. This is the first reported case of IDCT in Korea, which pathologists should include in the differential diagnosis of Langerhans cell-like lesions.

## CASE REPORT

A 29-year-old female patient presented with a 1.2-cm, ill-defined, erythematous subcutaneous nodule on the left flank (Fig. 1A), which appeared a month ago and increased in size over time. She had no cutaneous or extracutaneous symptoms. The patient was otherwise healthy with no remarkable medical history. Skeletal survey and bone scan revealed no bone lesions.

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Excision of the nodule was performed. Microscopically, the lesion showed poorly circumscribed infiltrates of tumor cells forming mostly solid sheets in the dermis and subcutis (Fig. 1B). No necrosis or adnexal invasion was noted. A small amount of peritumoral inflammatory cell infiltration, composed mostly of lymphocytes, was observed. Eosinophils were notably absent (Fig. 2A). Tumor cells showed monotonous morphology with indistinct cell borders. They had moderately abundant and lightly eosinophilic cytoplasm (Fig. 2B). The tumor cells had enfolded or reniform vesicular nuclei with inconspicuous nucleoli, and some of the tumor cells showed longitudinal nuclear grooves. Although occasional neoplastic cells had a large nucleus, mitotic figures were rarely seen (Fig. 2C). Characteristically, there was no epidermal involvement by tumor cells (Fig. 2D). The neoplastic cells in the tumor were diffusely immunopositive for S100 and CD1a (Fig. 3A, B). Langerin (CD207) was clearly negative in the tumor cells. Scattered langerin-positive bystander Langerhans cells serve as internal positive controls (Fig. 3C). Despite intensive scrutiny, no Birbeck granules were identified on electron microscopy (Fig. 3D).

## DISCUSSION

In this paper, we report a case of IDCT, an extraordinarily rare neoplasm. Most IDCTs occur in adults without predilection for either sex.[3,5] IDCT is almost always restricted to the skin without systemic symptoms.[2,6] Its rarity and resemblance to LCL often cause as diagnostic challenge for pathologists who are not familiar with this entity. Uniform expression of CD1a and S100 protein enables distinction of IDCT from other forms of non-Langerhans cell neoplasms, such as juvenile xanthogranuloma, xanthoma, histiocytic sarcoma, and reticulohistiocytosis.[7] The dif-





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ferential diagnosis also includes Langerhans cell lineage tumors, such as Langerhans cell histiocytosis (LCH) and Langerhans cell sarcoma (LCS). LCH is a rare condition and mostly occurs in childhood.[7] In cutaneous LCH, the lesions present as papules or plaques.[3] Tumor cells show typical morphology of Langerhans cells, which are indistinguishable from those of IDCT.[5] However, unlike IDCT, LCH shows evident epidermotropism with intraepidermal Langerhans cell microabscesses.[3] Presence of eosinophilic infiltration is another important difference from IDCT.[3] Immunopositivity for S100 protein, CD1a, and langerin (CD207), and the presence of Birbeck granules confirm the diagnosis of LCH.[2] Although LCH shows variable outcomes, most of the cases require long-term follow-up. LCS is another important tumor in the differential diagnosis. LCS is a very rare high-grade neoplasm.[8] Unlike in IDCT, epidermotropism and eosinophil infiltration are not usually evident in LCS;[5] however, most of the tumor cells show overtly malignant cytology including pleomorphic and hyperchromatic nuclei with frequent mitotic figures.[8] Langerhans cell differentiation of neoplastic cells of LCS must be confirmed by immunohistochemistry (IHC) or electron microscopy. LCS is an aggressive neoplasm and has very poor prognosis.[8] In the present case, langerin (CD207) immunonegativity of the tumor cells and the absence of Birbeck granules on electron microscopy were crucial for definitive diagnosis of IDCT.[5]

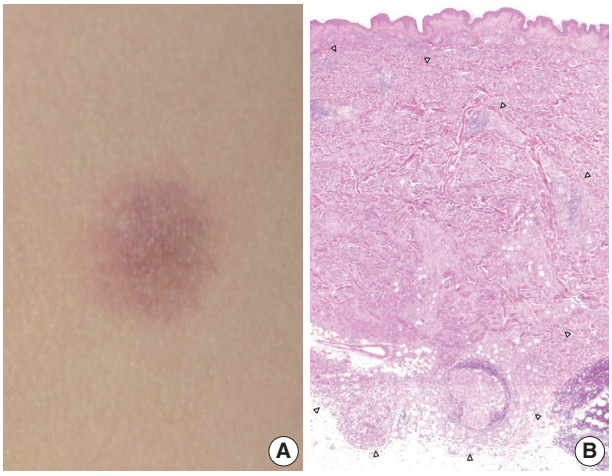


**Fig. 1.** (A) An ill-defined, 1.2-cm, round, erythematous subcutaneous nodule on the left flank. (B) The ill-defined tumor is mainly located in the dermis and subcutis. The margin of the tumor is marked by arrowheads.

Regarding the utility of IHC in comparison with electron microscopy, langerin IHC is important in the differentiating be-

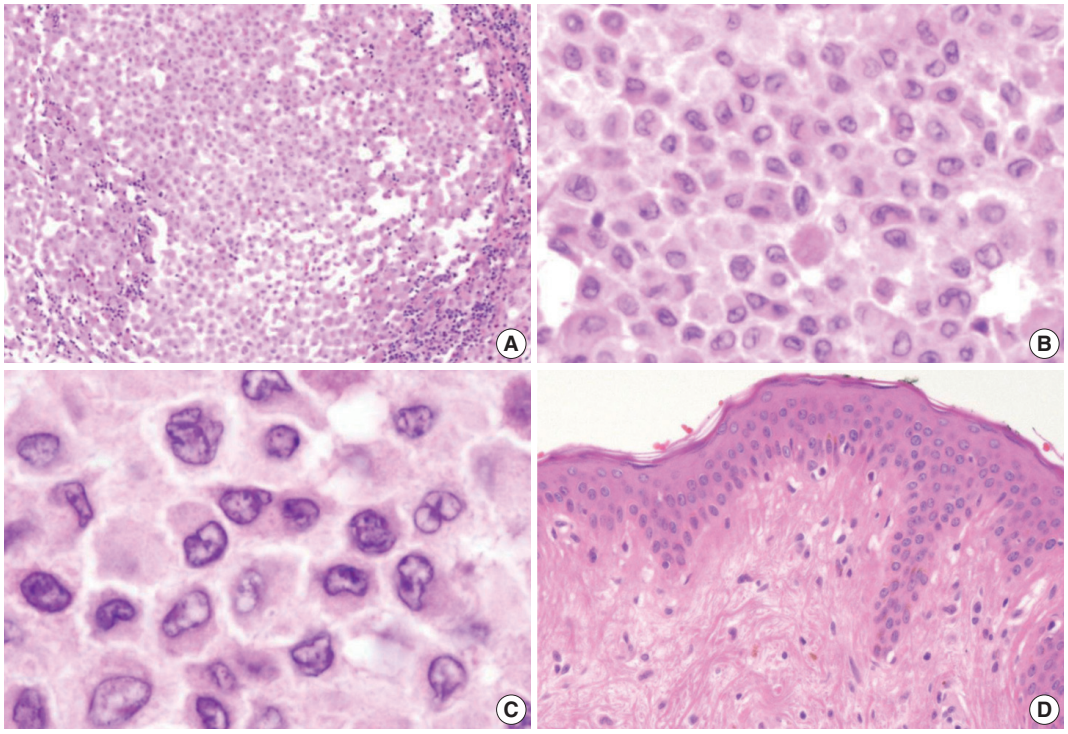


**Fig. 2.** (A) There is patchy infiltration by aggregates of the tumor cells. Tumor cells are usually monotonous and have indistinct cell borders. Clusters of lymphocytes are admixed with tumor cells in focal areas without eosinophils. (B) The constituent cells have ovoid cell morphology with abundant eosinophilic cytoplasm. (C) Their nuclei are oval and sometimes indented. Nuclear grooves are frequently seen. Although occasional enlarged nuclei are identified, mitoses are rarely seen. (D) There is no epidermal involvement.

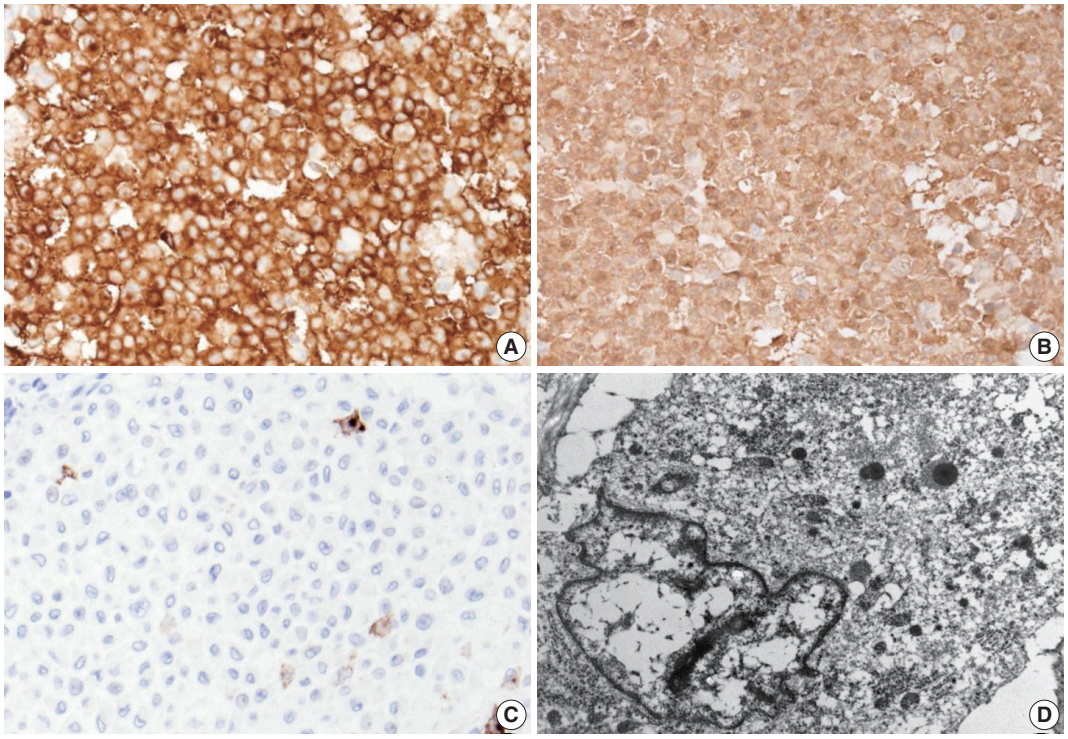


**Fig. 3.** The neoplastic cells diffusely express CD1a (A) and S100 protein (B). (C) Langerin (CD207) is typically negative. (D) No Birbeck granules are seen on transmission electron microscopy (×8,000).

tween true Langerhans cells and Langerhans cell-like lesions.[2] Langerin is an antibody to a transmembrane C-type lectin that associates with Birbeck granule formation. Previous studies revealed high sensitivity and specificity of langerin for Langerhans cell differentiation.[9,10]

In summary, IDCT should be included in the differential diagnosis for a lesion composed of Langerhans cell-like cells without epidermotropism and eosinophilic infiltration. To our knowledge, the present study is the first report of IDCT in Korean pathologic literature. Making an accurate diagnosis of IDCT is important to avoid overly aggressive management.

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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