HEPATOPANCREATOBILIARY PATHOLOGY

Co-expression of MST1R and MET Receptors and Distant Metastasis in Stage I Hepatocellular Carcinoma

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Background: The MST1R (RON) and MET receptors are tyrosine kinases that form a non-covalent complex on the cell surface that functions in several steps of invasive growth, including migration, cell dissociation, and matrix invasion. The purpose of this study was to determine the predictive or prognostic significance of MST1R and MET expressions in the prediction of distant metastases of stage I hepatocellular carcinoma (HCC) patients.

Methods: The correlation between expressions of MST1R and MET and patient outcome was retrospectively examined by immunohistochemistry in 144 stage I HCC patients, including 72 patients with distant metastases and 72 age-, gender-, date of surgery-matched control patients with no evidence of metastatic disease, using logistic and Cox models.

Results: Positive immunostaining for MST1R and MET was detected more frequently in the tumors of the metastasis group than in those of the control group (61.1% vs 38.9%, p = 0.012 and 29.2% vs 8.3%, p = 0.002, respectively). Multiple logistic regression analysis showed that co-expression of MST1R and MET was a significant independent predictor of distant metastasis (odds ratio, 7.709, p = 0.003). MST1R-positive patients had lower 10-year disease-free survival (DFS) rates than MST1R-negative patients (p = 0.008). MET-positive patients had lower 10-year DFS rates than MET-negative patients (p < 0.001). Multivariate Cox analysis identified co-expression of MST1R and MET as an independent prognostic marker for DFS, along with the type of surgery (hazard ratio, 2.739; p < 0.001).

Conclusions: Co-expression of MST1R and MET appears to predict an aggressive phenotype in early stage HCC patients.

Key Words: Carcinoma, hepatocellular; RON protein; MET; Metastasis; Prognosis

Expression of Serum Response Factor and Its Chemo-resistant Effect in Hepatocellular Carcinoma

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Background: The epithelial-mesenchymal transition (EMT) is a crucial process in tumor progression. EMT of tumor cells not only causes increased metastasis but also contributes to drug resistance. In this study, we examined the effect of EMT related serum response factor (SRF), focusing on its impact on chemo-resistance effect in hepatocellular carcinoma (HCC).

Methods: We investigated SRF and Snail expression in 146 cases of HCCs by immunohistochemistry. We examined the chemoresistant effect and role of SRF in HCC by transfecting the SRF cDNA in HLE cells and the SRF anti-sense cDNA in sarcomatoid HCC cells.

Results: Expression of SRF and Snail were detected in 37.6% (55 in 146) and in 12.3% (18 of 146) of the HCCs. None of the non-tumorous liver tissues showed SRF and Snail expression. SRF expression closely correlated with the expression of Snail (p < 0.001). Expression of SRF and Snail showed significant correlation with the high histologic grade (p = 0.015 and p = 0.003, respectively). Over-expression of SRF in HLE cells led to increased expression of mesenchymal markers, as well as increased cell growth and colony formation. Over-expression of SRF also led to significantly reduced cytotoxic effect of sorafenib in HLE cells. Conversely, inhibition of the SRF expression in the sarcomatoid SH-J1 cells by the SRF anti-sense cDNA significantly enhanced cytotoxic effect of sorafenib along with the attenuated expression of mesenchymal markers.

Conclusions: These results suggest that SRF is critical for HCC to acquire mesenchymal phenotype toward resisting sorafenib-mediated cellular injury.

Key Words: Carcinoma, hepatocellular; Serum response factor; Chemoresistance

Intraductal Tubular Adenoma, Pyloric Gland Type, of the Liver

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Pyloric type intraductal tubular adenoma (ITA) of the biliary tracts is very rare; only several cases arising in the extrahepatic bile duct have been reported in the literatures. Herein we present a first case of intrahepatic ITA, pyloric type. A 63-year-old male with a history of hepatocellular carcinoma with transsphenoid arterial chemoembolization (TACE) was admitted. On the follow-up computed tomography scan obtained 8 months after TACE, intraductal mass with heterogeneous enhancement in intrahepatic bile duct of segment 5 was observed. On magnetic resonance imaging with fat suppression, the intraductal mass exhibited low signal intensity on the T1-weighted image, and heterogeneous high signal intensity on the T2-weighted image. Surgical exploration revealed a 3 × 3 cm sized mass in a cystically dilated bile duct. The tumor consisted predominantly of closely packed tubular structures with areas of coagulation necrosis, suggesting the torsion of the lesion. The tubular structures were lined by cuboidal to columnar cells with clear to eosinophilic cytoplasm and basally located nucleus resembling pyloric glands. The immunohistochemical staining of the tumor revealed positive reaction for keratin7, keratin19, MUC6, and MUC5Ac but negative reaction for MUC1, MUC2, keratin20, CDX2, and TP53. On the basis of the histological and immunohistochemical findings, a final diagnosis of pyloric type ITA of the liver was made. Although more case studies of ITA in the biliary tracts are required for the study of tumorigenesis and pathologic features of this rare tumor, the lesion may be the intrahepatic biliary counterpart of pancreatic ITA.
Extrahepatic cholangiocarcinoma (ECC) is a malignant tumor of the biliary system with a poor prognosis. This poor prognosis is predominantly the result of cancer invasion and metastasis. Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that consists of an extracellular domain “EpEX,” a single transmembrane domain and an intracellular domain “EpICD.” Recent findings have shown that EpICD interacts with beta-catenin to translocate into the nucleus, induces cell proliferation, migration and invasion. **Methods:** We examined the expression and cellular localization of EpEX, EpICD, and beta-catenin in 79 cases of ECC and evaluated its relationship with clinicopathologic factors. In addition, we examined the role of EpCAM using synthetic small interfering RNA (siRNA) in silencing the EpCAM gene expression in cholangiocarcinoma cells. **Results:** EpEX was expressed in 78 of 79 (98.7%) cases of ECC, exclusively in the cell membrane. Nuclear translocation of EpICD and beta-catenin was present in 27.8% and 54% of specimens in cancer cells, respectively. No nuclear translocation of EpICD or beta-catenin was observed in non-neoplastic bile duct cells. Nuclear translocation of EpICD and beta-catenin was frequently observed in cancer cells of the invasive front and these expressions were significantly associated with the degree of tumor differentiation. There was also a strong correlation between nuclear translocation of EpICD and beta-catenin. Silencing the EpCAM by siRNA significantly decreased the cell proliferation, cell migration and inva-
Background: Distinguishing hepatocellular carcinoma (HCC), especially for well differentiated HCC, from benign hepatocellular lesions is a well-recognized problem in pathologic diagnosis. It is necessary to identify some novel marks for accuracy diagnosis of HCC. Methods: Paraffin blocks of HCC and adjacent liver cirrhosis tissues from 51 patients who underwent curative resection of HCC were used for this study. Serum HBsAg test was positive for these patients. The normal liver tissues were obtained from 15 serum HBsAg negative patients with liver angioma resection. Immunohistochemistry was applied to detect the expression and distribution of HBsAg, CD34, and alpha-fetoprotein (AFP). Results: In normal liver, hepatic sinusoids were always negative for CD34 and liver cells were negative for HBsAg and AFP. In HBV-associated cirrhosis, HBsAg was focally to diffuse positive and the CD34 were sparsely expressed in capillarized sinusoids at perportal and perinodular area in all cases. AFP was focally positive in 9.8% patients (5/51). In advanced HCC, only 3.9% HCC cases were focally positive for HBsAg (2/51). However, CD34 was strongly and diffusely expressed by the endothelial lining of sinusoid-like tumor vessels in 92.2% HCC cases (47/51). The focal CD34 staining pattern was seen in remaining 4 cases of HCC. Focal and diffuse AFP staining was observed in 39.2% patients (20/51). A significant difference was found between cirrhosis and HCC in HBsAg, CD34, and AFP staining. Conclusions: The absence of HBsAg immunoreactivity, in combination with a complete CD34 immunostaining pattern and diffuse AFP staining, greatly facilitates the pathologic diagnosis of HCC.

Key Words: Carcinoma, hepatocellular; Hepatitis B surface antigens; Antigens, CD34; Alpha-fetoprotein
Expression of Kinesin Family 5A in Hepatocellular Carcinoma: Role during Hepatocarcinogenesis

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Background: KIFs are a conserved class of microtubule-dependent motor proteins that have adenosine triphosphatase activity and motion characteristics. Active movement of kinesins supports several cellular functions, such as mitosis, meiosis, and transport of macromolecules. Abnormal expression and function of kinesins plays a key role in development or progression of many human cancers. In this study, we investigated expression of KIF5A in human hepatocellular carcinomas (HCCs) via immunohistochemistry and their relations with clinicopathological parameters, pursuing their roles in development and progression during hepatocarcinogenesis. Methods: Surgically resected HCCs from 58 patients were selected. Immunohistochemistry was performed with anti-KIF5A antibodies using auto-immunostainer. Results: Expression of KIF5A was detected at cytoplasm and occasionally membrane of HCC cells. Thirty-nine HCCs (67.2%) showed KIF5A expression and 58 HCCs regrouped into 37 low grade expression (63.8%) and 21 high grade (36.2%). Five HCCs (8.6%) showed ring shaped clumping, aberrantly. Higher KIF5A expression was not significantly associated with sex, age, high preoperative alpha-fetoprotein level, Child-Pugh classification, etiology, portal vein invasion, tumor size, presence of necrosis, differentiation, presence of capsule, septum formation, fatty change, vascular invasion and recur. HCCs with ring shaped clumping showed significant association with human papillomavirus (p = 0.0186), well differentiation (p = 0.0076), presence of fatty change (p = 0.0063). Univariate and multivariate analysis identified that KIF5A over-expressions was not an independent predictive factor for recurrence after operation. Conclusions: This study showed that over-expression of KIF5A did not suggest any relation with clinicopathological parameters or recurrence. However, aberrant ring shaped clumping pattern of KIF5A expression might be able to be an important factor, related with hepatocarcinogenesis.

Key Words: Hepatocellular carcinoma; KIF5A; Immunohistochemistry
ma to be a carcinoma with a sarcomatoid component. We report a case of undifferentiated spindle cell carcinoma of the gallbladder.

Key Words: Gallbladder neoplasms; Carcinoma; Sarcomatoid carcinoma

High Expression of Aldo-Keto Reductase 1B10 Is an Independent Predictor of Good Prognosis in Hepatocellular Carcinoma

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Background: Up-regulated aldo-keto reductase 1B10 (AKR1B10) through the mitogenic activator protein-1 signaling pathway might promote hepatocarcinogenesis and tumor progression. The goal of this study was to evaluate the prognostic significance of AKR1B10 protein expression in hepatocellular carcinoma patients. Methods: Tissue microarray was used to detect the expression level of AKR1B10 protein in tumor from 255 hepatocellular carcinoma patients undergoing curative hepatectomy. The impact of AKR1B10 expression on survival of patients was analyzed. Disease-specific death was defined as: tumor occupying more than 80% of the liver; portal venous tumor thrombus proximal to the second bifurcation; obstructive jaundice due to the tumor; distant metastases; or variceal hemorrhage with portal venous tumor thrombus proximal to the first bifurcation. Results: High AKR1B10 expression was observed in 125 (49.0%) of the 255 hepatocellular carcinomas. It was associated with no major portal vein invasion (p = 0.022), no intrahepatic metastasis (p = 0.010), lower American Joint Committee on Cancer (AJCC) T-stage (p = 0.016), lower Barcelona Clinic Liver Cancer (BCLC) stage (p = 0.006), and lower α-fetoprotein level (p = 0.020). High AKR1B10 expression was correlated with no early recurrence (p = 0.022), but not with the late recurrence (p = 0.255). Multivariate analyses of survival revealed that intrahepatic metastasis, lower albumin level, and liver cirrhosis were independent predictors of shorter recurrence-free survival. Intrahepatic metastasis and lower albumin level were independent predictors of shorter disease-specific survival. High AKR1B10 expression was an independent predictor of both longer recurrence-free survival (p = 0.024) and longer disease-specific survival (p = 0.046). Conclusions: High AKR1B10 expression might be useful as a marker for good prognosis in hepatocellular carcinoma patients after curative hepatectomy.

Key Words: AKR1B10; Carcinoma, hepatocellular; Recurrence; Survival; Prognosis

Survival in Pancreatic Cancer Patients with ≤ 2 cm and Extension beyond Pancreas Is Not Worse Than Those with > 2 cm and Limited to Pancreas

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Background: The American Joint Committee on Cancer (AJCC) T classification of the European Neuroendocrine Tumor Society (ENETS) guideline. Using a prospective pancreatic database, we investigated risk factors of lymph node metastasis (LNM) or post-operative recurrence. Results: There were stage I disease in 6 patients, stage IIa in 4, stage IIIa in 1, stage IIIb in 5, and stage IV in 2 patients. There were G1 in 6 patients, G2 in 8 patients, and G3 in 4 patients LNM was found in 5 out of 9 patients with tumors located in the pancreatic head (Ph) and in 1 out of 9 patients with tumors located at the pancreatic body/tail (Pbt). There was no LNM in patients with tumor size less than 15 mm. Post-operative recurrence (liver metastasis) was found in 2 out of 8 patients with G2 and 3 out of 4 patients with G3. Any metastasis as recurrence was not found out G1 group. Furthermore, Ph and tumor size > 15 mm or G2/G3 were risk factors for lymph node metastasis. Risk factor of recurrence was G2/G3 and tumor size > 15 mm. Conclusions: In patients with Ph tumor and tumor size > 15 mm or G2/G3, minimal invasive surgery should be avoided. Special attention for post-operative recurrence should be paid in patients with G2/3 and tumor size > 15 mm.

Key Words: pNET; Lymph node metastasis; Recurrence; Risk factor; Pancreatectomy

Clinicopathological Features of Patients with Non-functional Pancreatic Neuroendocrine Tumor Who Underwent Surgical Resection

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Background: The aim of this study is to investigate clinicopathological features of patients with non-functioning pancreatic neuroendocrine tumor (NFpNET) who underwent pancreatectomy. Methods: Eighteen patients with NFpNET underwent curative surgical resection between Nov 2004 and 2011. All patients were classified based on 2010 World Health Organization (WHO) classification criteria and TNM classification of the European Neuroendocrine Tumor Society (ENETS) guideline. Results: Among the 18 patients, G1 was in 6 patients, G2 in 8 patients, and G3 in 4 patients. LNM was found in 5 out of 9 patients with tumors located in the pancreatic head (Ph) and in 1 out of 9 patients with tumors located at the pancreatic body/tail (Pbt). There was no LNM in patients with tumor size less than 15 mm. Post-operative recurrence (liver metastasis) was found in 2 out of 8 patients with G2 and 3 out of 4 patients with G3. Any metastasis as recurrence was not found out G1 group. Furthermore, Ph and tumor size > 15 mm or G2/G3 were risk factors for lymph node metastasis. Risk factor of recurrence was G2/G3 and tumor size > 15 mm. Conclusions: In patients with Ph tumor and tumor size > 15 mm or G2/G3, minimal invasive surgery should be avoided. Special attention for post-operative recurrence should be paid in patients with G2/3 and tumor size > 15 mm.

Key Words: pNET; Lymph node metastasis; Recurrence; Risk factor; Pancreatectomy
Validation Study for T and N Classification of Distal Extrahepatic Bile Duct Carcinoma: According to Depth of Invasion and the Number of Metastatic Lymph Nodes

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Background: In current American Joint Committee on Cancer (AJCC) staging system, T stage of distal bile duct carcinoma is classified according to tumor extent within or beyond the bile duct wall. Alternative T stage by depth of invasion (DoI; T1, <5 mm; T2, 5 to 12 mm; and T3, >12 mm) has been proposed by Hong et al. In this study we validated new T stage by DoI in distal bile duct carcinoma. Methods: We evaluated the DoI depth in 114 cases of distal bile duct carcinoma through digital scan image. DoI was measured in two methods. First, DoI-1 was defined as distance between the basal lamina of the adjacent normal bile duct mucosa to the most deeply invasive tumor cells. Second, DoI-2 was measured as distance from top of tumor to the most deeply invasive tumor cells except for intraductal papillary neoplasm. Both data of DoI-1, 2 were compared patient’s survival. Results: T stage of current AJCC staging system showed poor correlation with patient’s survival. However both DoI-1, 2 showed good correlation with patient survival with statistical significances (p=0.0005 in DoI-1; p=0.0015 in DoI-2). Conclusions: New T classification using DoI, which is measured from the basal lamina of the adjacent normal bile duct or top of tumor to the most deeply invasive tumor cells, is more appropriate T classification system for distal bile duct carcinoma that showed good correlation with patient’s survival. DoI could be more practical and reliable method in T classification of distal bile duct cancer staging.

Key Words: Distal extrahepatic bile duct; Depth of invasion; Stage

Overexpression of Yes-Associated Protein 1 in Hepatocellular Carcinoma with Stemness-Related Markers and Combined Hepatocellular and Cholangiocarcinoma

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Background: Combined hepatocellular-cholangiocarcinoma (cHC-CC) and some hepatocellular carcinoma (HCC) express stemness-related markers, such as epithelial adhesion molecule (EpCAM) and keratin 19 (K19), and they have been reported to have more aggressive behavior compared to HCC without stemness-related markers, although the pathogenesis remains unclear. Yes-associated protein 1 (YAP1), a potential oncogene, is known to promote stem cell proliferation. The expression pattern of YAP1 protein and clinicopathological features were compared among HCCs with or without expression of stemness-related markers, and cHC-CCs. Methods: Sixty-four cases of HCCs expressing both of EpCAM and K19, 36 HCCs with double-negative expression of EpCAM and K19, and 58 cHC-CCs were studied. The expression patterns of YAP1 evaluated by immunohistochemistry and clinicopathological features were compared among three groups. Results: Nuclear YAP1 expression was significantly increased in HCC expressing stemness-related markers (20/36, p<0.001) and cHC-CC (39/58, p<0.001), respectively, than that of HCC with no expression of stemness-related markers. Increased nuclear YAP1 expression was correlated with poor histological differentiation in HCC expressing stemness-related markers and cHC-CC. Conclusions: Our study suggests that nuclear YAP1 expression is significantly increased in HCC expressing stemness-related markers and cHC-CC and YAP1 is a potential therapeutic target of the tumors.

Key Words: Hepatocellular carcinoma; Combined hepatocellular and cholangiocarcinoma; YAP

Grouping Based on Invasion Depth Is a Better Alternative Method Overcoming the 7th Edition of the American Joint Committee on Cancer Staging System for Distal Bile Duct Carcinomas: 281 Cases

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Background: The 7th edition of the American Joint Committee on Cancer (AJCC) staging system for distal bile duct cancer may not be accurate, because the current T classification did not consider actual invasion depth arising from middle and distal (intrapancreatic) common bile duct carcinomas. Methods: Two hundred eighty-one patients diagnosed as distal bile duct carcinomas between 1991 and 2004 from a single institution were included. Invasion depth of all cases were measured, the relationships between clinicopathological parameters and groups based on invasion depth (G1, <5 mm; G2, 5-12 mm; G3, >12 mm) were evaluated, and the survival time of each group based on invasion depth and T classification was compared. Results: The deeper invasion groups were more commonly associated with infiltrative growth pattern, higher histological grade, lymph node metastasis, pancreatic, duodenal, vascular, perineural invasions and worse overall survival (all p<0.05). The 7th T classification and stage grouping were unable to discriminate among T1, T2, and T3 lesions, while groups based on invasion depth and modified stage grouping by incorporating groups based on invasion depth and current N classifications (all p<0.05) could segregate more clearly than the 7th AJCC staging system. In multivari-
ate analysis, grouping based on invasion depth remained as one of the prognostic factors (p < 0.05). **Conclusions:** The grouping based on invasion depth of distal bile duct carcinomas is a better alternative method overcoming the current 7th AJCC staging system. Therefore, invasion depth should be measured and incorporated into pathologic assessment of distal bile duct carcinomas.

**Key Words:** Bile duct neoplasms; Extrahepatic; Invasion depth; Neoplasm staging; Survival

**Epithelial Cell Adhesion Molecule Expression in Hepatocellular Carcinoma: An Independent Unfavorable Prognostic Marker**

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**Background:** Epithelial cell adhesion molecule (EpCAM) has been proposed as a stemness-related marker and a WNT-beta-catenin signaling target gene in hepatocellular carcinoma (HCC). **Methods:** The expression of EpCAM in a tissue microarray of 227 primary HCCs has been examined by immunohistochemistry and correlated with clinico-pathological data and aberrant expression of beta-catenin. **Results:** EpCAM was expressed in 17.2% (39/227) and 19.2% (20/104) of primary HCCs in all and intermediate/advanced (pT2-T4) stages, respectively. In a subset of intermediate/advanced stage of HCCs, the expression of EpCAM was associated with younger patient age (p < 0.001), microscopic vascular invasion (p = 0.004) aberrant expression of beta-catenin (p = 0.009) and poorer prognosis (overall survival, p = 0.048; disease-free survival, p < 0.001). Moreover, EpCAM was an independent prognostic factor of unfavorable clinical outcome (overall survival: hazard ratio [HR], 2.42; p = 0.023 and disease-free survival: HR, 2.54; p = 0.024). **Conclusions:** EpCAM expression is useful in predicting unfavorable clinical outcome in HCC of intermediate/advanced stages.

**Key Words:** Intraductal papillary mucinous neoplasm; Microcystic, elongated and fragmented glandular features; Pancreas

**Hepatitis B Virus X Protein Upregulates SRC Homology 2-Containing Protein Tyrosine Phosphatase (SHP2) Expression via the Nuclear Factor-κB Pathway in Hepatocellular Carcinomas**

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**Background:** Hepatitis B virus X protein (HBX) has a crucial role in the development of hepatocellular carcinoma and activates many transcription factors, including AP-1, nuclear factor-kB (NF-kB) and CREB. SRC homology 2-containing protein tyrosine phosphatase (SHP2) is a signal transducer of the EGFR-RAS-RAF-MAPK pathway. **Methods:** In the present study, we examined the SHP2 expression in thirty cases of hepatitis B virus (HBV)-positive hepatocellular carcinomas by Western blot analysis. To investigate the links between HBX and SHP2 expression, the activities of the SHP2 promoter were analyzed using luciferase assays, chromatin immunoprecipitation-real time polymerase chain reaction assays and Western blotting. We further investigated the relationship of SHP2 and HBX in stably HBX-overexpressing Huh7-X and transiently HBX expressing HEK293 cells. **Results:** The level of SHP2 proteins was concomitantly increased with HBX in a dose-dependent manner in HEK293 cells. We identified a putative NF-kB binding site within 500 bp of the SHP2 promoter and also observed the decrease of SHP2 proteins after the treatment of the NF-kB/p65 inhibitor, parthenolide in HBX expressing cell. In addition, we confirmed that the complex of NF-kB and HBX proteins direct bound to the SHP2 promoter. **Conclusions:** We conclude that HBV infection induces SHP2 overexpression through the HBX-NF-kB pathway and the amplification of SHP2 expression is dependent in the complex of NF-kB and HBX proteins.

**Significance of Microcystic, Elongated and Fragmented Glandular-like Feature of Intraductal Papillary Mucinous Neoplasm of the Pancreas**

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**Background:** Microcystic, elongated and fragmented (MELF) glandular features are associated with epithelial-mesenchymal transition, tumor invasion, and progression in endometrial carcinomas. Similar histologic features are also observed at the periphery of pancreatic intraductal papillary mucinous neoplasms (IPMNs). However, clinicopathologic significance of MELF-like features has not been studied in IPMNs, whether MELF-like features may represent a regenerative change to ruptured neoplastic epithelia or early microscopic features of stromal invasion. **Methods:** To address this question, MELF-like pattern was assessed in the surgically resected 154 IPMN cases (118 noninvasive IPMNs and 36 IPMNs associated carcinomas) and compared the data with clinicopathologic factors. MELF-like patterns were defined as neoplastic glandular appearance showing dilatation, elongation, fragmentation with fibromyxoid stromal reaction, and/or inflammation at the periphery of IPMNs. **Results:** MELF-like features were observed in 50 IPMN cases (low grade dysplasia, 6/55 cases, 10.9%; intermediate grade, 12/50, 24.0%; high grade, 6/13, 46.2%; associated carcinoma, 26/41, 72.2%). MELF-like features were associated with higher degree of dysplasia (p < 0.0001) and larger tumor size (p = 0.009). But, MELF-like features were not correlated with age, sex, tumor location, subtypes based on the involved pancreatic ducts (main or branch duct). When survival of the patients were compared, no survival difference was observed in noninvasive IPMN patients with or without MELF-like features (p = 0.849). **Conclusions:** MELF-like feature are more commonly observed as the degree of dysplasia and tumor size increased. Further studies are needed to characterize MELF-like features in IPMNs whether it may represent a regenerative change to ruptured neoplastic epithelia or early microscopic features of stromal invasion.

**Key Words:** Intraductal papillary mucinous neoplasm; Microcystic, elongated and fragmented glandular features; Pancreas
tion of the EGFR-RAS-RAF-MAPK signal by SHP2 may promote tumor cell proliferation.

**Key Words:** Carcinoma, hepatocellular; SHP2; Hepatitis B virus X protein; NF-kappa B; Hepatitis B virus

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**AP14-PP-0024**

H3K27ac and Its Correlation with H3K27me3 in Hepatocellular Carcinoma Using Objective-Quantitative Immunohistochemical Method

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**Background:** Hepatocellular carcinoma (HCC) is a major cancer worldwide. Genomic studies of cancers including HCC revealed that epigenetic changes as well as genetic changes are important for carcinogenesis. Histone modifications and their associated enzymes have been regarded as important targets for new cancer drugs. Immunohistochemical studies using tissue samples and clinicopathological data are potentially useful to find clinicopathologically significant histone modifications. One of problems, however, is how to ensure objectivity in evaluation of immunohistochemical results. **Methods:** Among various histone modifications, we especially focused on acetylation of lysine 27 on histone H3 (H3K27ac) and its correlation with tri-methylation of lysine 27 on histone H3 (H3K27me3). Neoplastic and non-neoplastic tissues of 198 HCC cases were immunostained with anti-H3K27ac and anti-H3K27me3 antibodies, and the images were analyzed by image analyzing program (Tissue Studio). The results were output as histological scores calculated by multiplying positive percentage of cells (0-100%) by the classified IHC marker intensity (0 to 3; range, 0 to 300). **Results:** HCC tissues showed significantly higher scores of H3K27ac levels compared to the back ground livers (p<0.001). HCCs showing high H3K27ac/high H3K27me3 (n=54) have association with poor differentiation (p<0.01) and the patients showed poor prognosis (p<0.01). Using confocal microscope, H3K27ac was present in central euchromatin regions, while H3K27me3 in peripheral heterochromatin regions. **Conclusions:** Concurrent activation of acetylation and tri-methylation in H3K27 occurs in poorly-differentiated HCC, and serves as an inverse prognostic marker in HCC patients. Quantitative image analysis used in this study is feasible and useful in evaluating histone modification by immunohistochemistry.

**Key Words:** Hepatocellular carcinoma; H3K27ac; H3K27me3; Immunohistochemistry

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**AP14-PP-0026**

Immunohistochemical Study of Intestinal Differentiation in Oncocytic-Type Intraductal Papillary Mucinous Neoplasms of Pancreas

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**Background:** On the basis of histopathological characteristics and immunohistochemical profiles of mucin core protein (MUC), intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are subclassified into four subtypes: gastric type, intestinal type, pancreatobiliary type and oncocytic type. Among these subtypes, MUC2, an intestinal-type mucin, has been considered to be a specific marker for intestinal-type IPMN. We investigated intestinal differentiation in oncocytic-type IPMNs of the pancreas and bile duct. **Methods:** We collected 6 cases of oncocytic-type IPMNs, 3 of the pancreas and 3 of the bile duct. Immunohistochemical expression of MUC1, MUC2, MUC5AC, and CDX2 was evaluated in these cases in addition to histopathological characteristics. **Results:** Three of the cases (1 of the pancreas and 2 of the bile duct) were focally positive for MUC2 while all the 6 cases were negative for CDX2 even in the MUC2-positive components. All the cases were positive for MUC5AC. Only 1 case of the pancreas was positive for MUC1. **Conclusion:** In this study, we revealed that a proportion of oncocytic-type IPMNs showed intestinal differentiation represented by MUC2 expression. On the other hand, none of oncocytic-type IPMN showed CDX2 expression, which was clearly different from intestinal-type IPMN. The results indicate that oncocytic-type IPMN is distinct from intestinal-type IPMN, but it could show intestinal differentiation in part.

**Key Words:** Intraductal papillary mucinous neoplasms; Oncocytic type; IOPN; MUC2; CDX2

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**AP14-PP-0025**

Aberrant AGR2 Expression Predicts Worse Patient Outcome in Poorly Differentiated Pancreatic Ductal Adenocarcinomas

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**Background:** Anterior gradient-2 (AGR2) protein is upregulated in various types of human cancer and its involvement in a malignant progression of pancreatic cancer has been suggested. The study aimed at determining the AGR2 expression in pancreatic ductal adenocarcinomas (PDACs) and evaluating AGR2 as a potential independent prognostic factor. **Methods:** The expression of AGR2 was immunohistochemically examined in tissues of 135 surgically resectable PDACs, semiquanti-

**Key Words:** Pancreas

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Combined Stromal Smoothened and Tumoral Sonic Hedgehog Overexpression as a Prognostic Factor in Extrahepatic Cholangiocarcinoma

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Background: Cross-talks between cancer and stromal cells are important for tumor growth of many carcinomas including cholangiocarcinoma and hedgehog signaling plays a key role in this interaction. Methods: We performed immunohistochemistry of 3 hedgehog signaling proteins, smoothened (SMO), patched1 (PTCH1), and sonic hedgehog (SHH), with 227 extrahepatic cholangiocarcinoma (EHCC) cases and compared clinicopathologic factors, including patients’ survival. Results: Stromal SMO and tumoral SHH overexpression was found in 70% (145/208) and 28% (62/220), and loss of tumoral PTCH1 expression was noted in 57% (127/221). Stromal SMO overexpression (p = 0.021) and loss of tumoral PTCH1 expression (p = 0.043) were associated with deeper cancer invasion. Combined stromal SMO and tumoral SHH overexpression was related with worse patients’ survival both by univariate (p = 0.001) and multivariate (p < 0.001) analyses. Conclusions: Combined stromal SMO and tumoral SHH protein overexpression can be used as a prognostic factor and stromal cells may be a therapeutic target for SMO antagonists in EHCCs.

Key Words: Bile duct, extrahepatic; Cholangiocarcinoma; SMO protein; SHH protein

Localized Mass-Forming IgG4-Related Autoimmune Pancreatobiliary Disease

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IgG4-related autoimmune disease may involve many organs, and it is histologically characterized by a dense lymphoplasmacytic cell infiltration with immunohistochemical and/or serologic IgG4 expression. IgG4-related autoimmune disease usually presents as diffuse organ involvement, especially in pancreatobiliary tract. However localized mass-forming autoimmune disease is rare. Here we report two localized mass-forming IgG4-related autoimmune disease of pancreas and bile duct, those were considered as pancreatic cancer and hilar cholangiocarcinoma. A 61-year-old male underwent trisectionectomy under impression of hilar cholangiocarcinoma. The resected specimen showed an ill-defined mass-like lesion on bile duct at hepatic bifurcation. Histologic examination revealed dense lymphoplasmacytic infiltration with fibrosis without malignant cells. Immunohistochemical stain demonstrated IgG4-positive plasma cells infiltration. A 61-year-old male patient received sur-
Surgery under impression of pancreas body cancer. The resected specimen revealed ill-defined mass-like lesion in pancreas body. Histopathologic examination showed locally dense lymphoplasmoid cell infiltration and numerous lymphoid follicles with fibrosis and accompanied low-grade intraductal papillary mucinous neoplasm without malignant cells. The plasma cells were positive for IgG4 immunostain. Localized mass-forming autoimmune disease is rare and difficult to differentiate in pre-operative evaluation. Although it is rare, localized mass-forming autoimmune disease should be considered in differential diagnosis of pancreatobiliary cancer because it is medically curable disease. We reported unusual two localized mass-forming IgG4-related pancreatitis and cholangitis those mimics pancreatic cancer and hilar cholangiocarcinoma.

Key Words: Mass-forming; IgG4; Autoimmune diseases

**High Recurrence of Transarterial Chemoembolization-Treated Hepatocellular Carcinoma with Stemness Post Liver Transplantation**

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**Background:** Cancer stem cells (CSCs) of hepatocellular carcinoma (HCC) expressing stem cell markers, such as K19 and EpCAM, have resistance for therapy and ability of seeding new tumors, therefore CSCs are suggested to be responsible for recurrence of HCC after liver transplantation (LT). Moreover, transcatheter arterial chemoembolization (TACE), which is used for therapeutic downstaging might select for or induce more aggressive tumor. The aim of this study was to determine if TACE treated HCCs have increased CSCs and whether CSCs are related to tumor recurrence after LT. **Methods:** The clinicopathological evaluation of 87 HCC patients who underwent LT was obtained. There were 35 cases with non-TACE and 52 cases with TACE. The expression of EpCAM, and cytokeratin 19 (CK19) was evaluated by immunohistochemistry. **Results:** In TACE group and non-TACE group, EpCAM was expressed in 21 (40.3%) and 9 (35.7%), and CK19 was found in 15 (28.8%) and 2 (5.7%), respectively. The expression of EpCAM and CK19 was higher in HCCs with TACE than those with non-TACE (p = 0.028 and p = 0.010, respectively). The recurrence rate of HCC was 5.7% in non-TACE group and 15.4% in TACE group. In TACE group, HCC patients with recurrence showed significantly higher expression of EpCAM and CK19 than those without recurrence (p = 0.036 and p = 0.040, respectively), whereas such difference was not found in non-TACE group. **Conclusions:** Increased expression of EpCAM and CK19 in TACE treated HCCs was related to the recurrence after LT. It is suggested that TACE might select CSCs of HCC, which have more aggressive behavior.

Key Words: Carcinoma, hepatocellular; Neoplastic stem cells; K19; EpCAM; Recurrence