MOLECULAR PATHOLOGY

The c-Myb-Induced Resistance of Colon Cancer Cells to Cisplatin Is Mediated by the p38 Mitogen-Activated Protein Kinase

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Background: The c-Myb transcription factor is essential for maintenance of stem-progenitor cells in colon epithelia and its expression is deregulated in premalignant adenomatous polyps and colorectal carcinomas. Patients with colorectal carcinoma exhibiting upregulated c-Myb expression have poor prognosis. This result can from low sensitivity of cancer cells to cytotoxic agents. Methods: In our study, increased resistance of colon carcinoma CT26 and HCT116 cells overproducing exogenous c-Myb to cytotoxic agents was observed. These cells lost sensitivity to cisplatin and doxorubicin upon upregulation of c-Myb. The effect of c-Myb can be mediated by the mitogen-activated protein kinase (MAPK) signaling since phosphorylation of the MAPKs p38 and JNK was enhanced in cells exhibiting high expression of exogenous c-Myb. Results: To determine functional contribution of these kinases to resistance of the c-Myb-overexpressing cells to cisplatin, we used BIRB796 to inhibit activity of p38 and SP600125 to inhibit JNK. We found that the c-Myb-induced resistance of CT26 cells to cisplatin can be reversed by BIRB796. Conclusions: This result documents that it is the p38 kinase that mediates the effects of c-Myb on sensitivity of colon cancer CT26 cells to cisplatin (This study was supported by grant NT13441/2012 of the Internal Grant Agency of the Ministry of Health, Czech Republic).

Key Words: Proto-oncogene proteins c-myb; Colonic neoplasms; Cisplatin; Resistance

Expression of CXCR4/SDF-1 Axis in Sarcoma Cell Lines

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Background: CXCR4 is chemokine-G protein coupled-receptor, which can play an important role in many biological processes. At present, the only ligand of CXCR4 was described: stromal cell-derived factor 1 (SDF-1). The CXCR4/SDF-1 axis is crucial for migration of stem cells in the organism. This pathway is also involved in metastasizing of tumor cells into organs with high levels of SDF-1. Recently, the expression of CXCR-4 is considered to be a marker of cancer stem cells in various types of solid tumors including sarcomas. Methods: Ten cell lines derived from the tumor tissue of patients treated for frequent types of sarcomas (5 osteosarcomas, 2 embryonal rhabdomyosarcomas, and 3 alveolar rhabdomyosarcomas). Furthermore, 3 other rhabdomyosarcoma cell lines were obtained from xenograft tumors developed in NOD/SCID mice after injection of embryonal rhabdomyosarcoma cells. CXCR4 and SDF-1 transcripts were detected using reverse transcription polymerase chain reaction, expression of CXCR4 protein was confirmed using indirect immunofluorescence. Results: Elevated levels of CXCR4 transcript were detected in all osteosarcoma cell lines if compared with rhabdomyosarcoma cell lines; however, these levels varied among individual cell lines. On the contrary, all examined cell lines showed high expression of SDF-1 transcript. Using immunodetection, we confirmed that CXCR4 showed predominantly membranous positivity, visible as a dotted signal on the cell surface. Conclusions: Detailed information concerning the expression of CXCR4/SDF-1 axis in sarcoma cells represents an important base for our next studies of CXCR4 co-expression with other cancer stem cells markers in sarcomas. Furthermore, our results bring the first evidence of CXCR4 expression in rhabdomyosarcomas.

Key Words: CXCR4; SDF-1; Neoplastic stem cells; Osteosarcoma; Rhabdomyosarcoma

The Dual Phosphatidylinositol 3-Kinase and mTOR Inhibitor NVP-BEZ235 Exhibits Anti-proliferative Activity and Overcomes Bortezomib Resistance in Mantle Cell Lymphoma Cells

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Background: Mantle cell lymphoma (MCL) is one of the most difficult B-cell lymphomas to be treated. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is constitutively activated in MCL and plays a critical role in tumor growth and survival. However, single targeted agent mTOR has limited efficacy in treating MCL. Here, we investigate for the first time potential efficacy of NVP-BEZ235 (BEZ235) in treating MCL by simultaneously targeting Akt and mTOR. Methods: Phosphorylated Akt and mTOR level were elevated in tissue samples from MCL patients and in MCL cell lines. We also generated bortezomib-resistant MCL cell lines and found increased phosphorylation of Akt and mTOR. Results: Individual inhibition of PI3K or mTOR had limited anti-proliferative effects, whereas dual inhibition with BEZ235 effectively inhibited cell growth. The effect of BEZ235 was synergistic and sensitized the cells to the cytotoxic effects of conventional agents. Furthermore, BEZ235 could overcome acquired resistance to bortezomib in MCL cells and suppress the activated Akt/mTOR pathway. Conclusions: These data suggest that the Akt/mTOR pathway plays a key role in the growth and survival of MCL cells and that these proteins may need to be simultaneously targeted for effective treatment of the disease. Our findings suggest that BEZ235 may be an effective agent for the treatment of MCL.

Key Words: Lymphoma, mantle-cell; Dactolisib; PI3K/mTOR
Proteomic Analysis of the Human Aortic Media

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Background: Arterial aging causes stiffening and luminal dilatation of the elastic arteries such as aorta. “Age-related medial degeneration and sclerosis” is the pathological term of arterial aging, and its histopathological findings include decrease and disruption of elastic fibers, increase of collagen fibers, increase of glycosaminoglycans, degeneration and loss of smooth muscle cells (SMCs) and microscopic calcification of the extracellular matrix in the arterial media. To characterize the molecular biology of arterial aging, we performed the proteomic analysis of the human aorta. Methods: Aortic samples were collected from 12 patients. Two patients were extremely old (100 and 96 years old), and two patients suffered from aortic dissection (91 and 79 years old), and eight control patients were without aortic aneurysms, Marfan syndrome or other connective tissue disorders. The aortic media were isolated and its proteome was analyzed with the use of two-dimensional polyacrylamide gel electrophoresis and mass spectrometry (matrix-assisted laser desorption/ionization-time of flight [MALDI-TOF] mass spectrometry [MS] and MALDI-TOF tandem mass spectrometry [MS/MS]). Results: The spots of vimentin of low molecular weight and low pI increased with aging. Several serum or secretory proteins (vitronectin, fibromodulin, alpha-1-antitrypsin, and antithrombin III) increased with aging. Conclusions: Modification of vimentin (an important intermediate filament of SMCs) may affect the function of SMCs or result from the degeneration of SMCs. Increased serum or secretory proteins may be related to the disorganization of extracellular matrix in arterial wall.

Key Words: Aging; Arteries; Proteome; Aorta

A Single-Tube, Multiplexed, Transcript-Based Assay to Detect ALK, ROSI and RET Fusions in Lung Cancer

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Background: Oncogenic fusions involving anaplastic lymphoma kinase (ALK), ROSI, and RET define a small subpopulation of non-small cell lung carcinomas (NSCLC). Tumors harboring ALK or ROSI gene fusions are sensitive to crizotinib whereas RET inhibitors may be of potential benefit for the treatment for RET-fusion positive tumors. Current methods for detection of ALK, ROSI, and RET fusions require multiple tissue samples and separate experiments. To explore a more practical screening modality, we developed a transcript-based assay for the simultaneous detection of the three fusions in lung cancer. Methods: A multiplexed, digital assay was designed to detect for presence or absence of fusion transcripts using NanoString’s gene expression technology. Utilizing a combined 3’ over-expression and fusion-specific detection strategy, we evaluated the performance of the single-tube assay on cell lines and clinical samples. Results: We successfully validated the assay in 264 NSCLC specimens and show that the assay is highly sensitive and specific. For ALK, our results were highly concordant (100% and 97.2%, respectively) to prior results obtained by fluorescence in situ hybridization (n=52) and immunohistochemistry (n=180). We identified six ROSI and twelve RET fusion-positive tumors and confirmed fusion status by reverse transcription polymerase chain reaction followed by sequencing. ROSI and RET fusions were significantly enriched in tumors negative for alterations in KRAS/EGFR/ALK. ALK/ROS1/RET fusions and EGFR/KRAS mutations were mutually exclusive to each other. Conclusions: As a single-tube test, our assay show promise for use in research and clinical practice given its practicality as a screening test for detection of the three rare but targetable fusions.

Key Words: Anaplastic lymphoma kinase; ROSI; Proto-oncogene proteins c-ret; Lung neoplasms; Diagnostics

Combined Genomic and Transcriptomic Analysis on Sorafenib-Sensitive and Resistant Cell Lines Reveals the Candidate Biomarker for Sorafenib-Responsiveness

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Background: Sorafenib is the only approved targeted therapy for hepatocellular carcinoma but it has been known that its effect to patients’ survival gain is limited as varying over a wide range depending on patho-genetic conditions. Thus, finding predictive markers for sorafenib-responsiveness and enhancing sorafenib sensitivity are essential for achieving efficient control of intractable hepatocellular carcinomas. Methods: Using high-throughput data including genomics and transcriptomics of 491 cancer cell lines from Cancer Cell Line Encyclopedia (CCLE), we tried to investigate the responsible molecules and corresponding pathways for sorafenib-responsiveness. We classified 491 cancer cell lines according to sorafenib responsiveness based on cell line response data from CCLE and performed differential expressed genes (DEGs) analysis between each groups. Also, gene ontology (GO) analysis and pathway-enrichment analysis were done. Some markers that have been acquired were tested on HCC patients’ tissues for sorafenib-responsiveness and survival analysis. Results: GO analysis reveals that sorafenib-sensitive cancer cells have upregulated immune response-related gene modules while resistant cells upregulated cellular adhesion and vessel development gene groups. Pathway enrichment analysis shows that extracellular matrix-receptor interaction and adhesion pathways are significantly activated in sorafenib-resistant pheno-
types. The predictive power of carefully selected markers among these molecules are under investigation. **Conclusions:** Systematic analysis on public big data can provide clues to prediction for sorafenib-responsiveness.

**Key Words:** Carcinoma, hepatocellular; Sorafenib; Predictive biomarker; Genome; Transcriptome

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**AP16-PP-0013**

**The Expressions of Cancer-Testis Antigen and Survivin with Demethylation in the Adipocyte-Derived Adult Stem Cell and K562 Cancer Cell Lines**

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**Background:** Cancer-testis antigen gene (CTAs) family shows cancer specific expression in adult cells. Some of their expressions are suspected to involve the anti-apoptosis and development from the embryonic stage. Recently, there have been efforts to find the common characteristics in stem cells and cancer cells. This study attempted to investigate the expressions of CTAs and survivin in adipocyte-stem cell (ASC) and K562 cancer cell lines with demethylating agent, 5-aza-2'-deoxycytidine (ADC). **Methods:** Reverse transcription polymerase chain reaction was performed to evaluate the expressions of surviving, and CTAs (GAGE 1-9, TRAG-3, MAGE-A1 to-A10) with ADC treatment. **Results:** In ASC, MAGE-A3, -A4, -A10 showed activation of their expressions and MAGE-A2 increased their expressions after treatment with ADC. Their maximal level of expressions showed at 48 hours after ADC treatment. However, MAGE-A1 and GAGE 1, 2, 8 was not detectable before and after ADC treatment. TRAG-3 and GAGE 3-7 were expressed in ASC before ADC treatment. In K562 cell lines, the expression of MAGE-A2, -A3, -A4, -A10, and TRAG-3 showed before ADC treatment and their expressions upregulated after treatment with ADC. The level of survivin mRNA was gradually decreased after exposure to ADC at time of 24 hours, 48 hours, and 72 hours in ASC, while its level was gradually increased in K562 cell lines after treatment with ADC. **Conclusions:** These results suggest that MAGE-A2, -A3, -A4, -A10 and TRAG-3 have similar properties regarding as methylation in K562 and ASC. However, survivin may have the different role in the demethylating status of K562 and ASC cells.

**Key Words:** Decitabine; Cancer-testis antigen; Survivin; K562 cell lines; Adipose stem cell

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**AP16-PP-0014**

**Molecular Differences within HER2 Immunohistochemistry-Equivocal and Fluorescence In Situ Hybridization-Amplified Breast Cancers**

**Mi Jung Kwon · Boin Lee · Myung-Ju Ahn · Jhingook Kim · Jin Seok Ahn · Keunchil Park · Ju Jin Kim · Seung Eun Lee · Taeceun Kim · Joungho Han · Yoon-La Choi**

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**Background:** Equivocal human epidermal growth factor receptor 2 protein immunohistochemistry 2+ (IHC-equivocal) results are found in 15-48% of breast cancers. Despite the targeted therapy according to fluorescence in situ hybridization (FISH) amplification, only 35% of patients with FISH-amplified, IHC-equivocal breast cancers respond to trastuzumab therapy. These results represent the genetic diversity within IHC-equivocal, FISH-amplified breast cancers. The aim of our study was to investigate the molecular differences within IHC-equivocal/FISH-amplified breast cancers and to determine whether there may be a correlation with the clinical outcome. **Methods:** We used quantitative real-time reverse-transcription-polymerase chain reaction (qRTPCR), quantitative real-time (qPCR), and multiplex ligation-dependent probe amplification (MLPA) assays in 55 IHC-equivocal breast cancers. **Results:** The high qPCR subgroup (HER2+/γ-actin gene copy number ratio >2.2) showed higher mRNA levels than the low qPCR subgroup (≤ 2.2) in IHC-equivocal/FISH-amplified breast cancers. The low qPCR subgroup with FISH- amplification showed similar mRNA levels to FISH-nonamplified/IHC-equivocal breast cancers. **Conclusions:** The combined analyses using mRNA expression, qPCR, and MLPA helped to separate the heterogeneous IHC-equivocal/FISH-amplified breast cancers into more detailed molecular categories.

**Key Words:** Breast neoplasms; ERBB2 protein, human; Real-time polymerase chain reaction; mRNA quantification; Multiplex polymerase chain reaction

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**AP16-PP-0015**

**Fibroblast Growth Factor Receptor 1 Amplification as a Druggable Target in Lung Squamous Cell Carcinoma**

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**Background:** Focal amplified gene is a potential target for diagnosis and therapy in cancer. Fibroblast growth factor receptor 1 (FGFR1) amplification has been known in squamous cell carcinoma of the lung (LSCC) and considered as a candidate gene for treatment and pathogenesis. The
aim of this study was to simultaneously explore the copy number changes of FGFR1 and the other 23 genes by multiplex ligation-dependent probe amplification (MLPA), and to compare fluorescent in situ hybridization (FISH), and to determine the clinicopathological significances.

**Methods:** The 81 resected LSCC specimens between 2008 and 2010 and 14 cell lines were enrolled. MLPA was performed in fresh tumor samples and cell lines and FISH was applied in formalin-fixed, paraffin-embedded tissues. **Results:** In MLPA, MYC (48.1%), AURKA (44.4%), MET (29.6%), KIT (21.0%), and FGFR1 (14.8%) showed amplification/gain. Twenty-two LSCCs (27.2%) had FGFR1 amplification in FISH (cut-off value ≥ 5). The concordance between FISH and MLPA was high (85.2%). However, no association between FGFR1 amplification and any clinicopathological variables was observed. Associations of epidermal growth factor receptor with FGFR1 FISH-amplified tumors, platelet-derived growth factor receptor A (PDGFRα) with poorly differentiation, KDR, ABL1, and MET with age, SMO and CCND2 with pT stages were identified. **Conclusions:** MLPA and FISH showed FGFR1 amplification was a common event occurring at 15-27.2% of LSCC. Amplified other genes may be also candidate target for LSCC.

**Key Words:** Receptor, fibroblast growth factor, type 1; Lung neoplasms; Carcinoma, squamous cell; Gene amplification; Multiplex polymerase chain reaction

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**AP16-PP-0014**

Improve Accuracy and Sensitivity of Detecting Epidermal Growth Factor Receptor Mutations by Direct DNA Sequencing Test

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**Background:** In Taiwan, high percentage of patients with non-small cell lung cancer (NSCLC) has epidermal growth factor receptor (EGFR) activating mutations. Those patients have well responses to EGFR inhibitors, such as Tarceva. To increase patients’ overall survive rate, an effective molecular diagnostic method for accurately detecting mutations and eliminating false positive results is needed. In the setting of more limited lung cancer specimens, such as biopsies and cytology, enrichment of target gene is critical to provide timely and correctly molecular diagnosis before treatment. **Methods:** Seventy three NSCLC patients were sampled (20 ng, genomic DNA) using a universal genetic detecting method (FemtoPath). Our results showed that FemtoPath/direct sequencing test identified EGFRM/K-rasW in 10 (43.48%) of the 23 tumors, EGFRV/K-rasM in 2 (8.69%) of the 23 tumors and EGFRM/K-rasM in 11 (47.83%) of the 23 tumors. Patients having both EGFR and K-ras mutations in genotype exon 19 del/K-rasM or L858R/K-rasM or S768N/K-rasM or V769M/K-rasM were separately four, seven, one and one in occurrence. Other rare EGFR mutations were also detected: three M766I, one T790A, one A767T, one Q791I, one S720F, one A767V, and one K860I. **Conclusions:** Rate of K-ras mutation and combinations of EGFRM/K-rasM mutations highly appear in NSCLC patients of Taiwan. Our results obtained by direct sequencing test could provide more information for understanding the correlation between the reactions of NSCLC patients to TKI therapies and genotype, especially in combinations of mutations.

**Key Words:** Genes, ras; Receptor, epidermal growth factor; Carcinoma, non-small-cell lung; Direct sequencing test

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**AP16-PP-0017**

Combinations of Epidermal Growth Factor Receptor and K-ras Mutations Rate Highly Appeared in Non-small Cell Lung Cancer Patients of Taiwan

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**Background:** Mutations of epidermal growth factor receptor (EGFR) and K-ras play an important role in the pathogenesis of lung cancer. Non-small cell lung cancer (NSCLC) patients with EGFR mutations or K-ras mutations in Asian are respectively 35% and 13%. EGFR mutations in NSCLC patients have good response to tyrosine kinase inhibitors (TKI) therapies and associated with longer overall survival. Some reports indicate that combinations of EGFR and K-ras mutations are rarely found in NSCLC patients. Besides, combinations of EGFR and K-ras mutations in NSCLC patients show positive initial response to TKI therapy or no response to TKI therapy. **Methods:** Twenty three NSCLC patients were sampled (20 ng, genomic DNA) using a universal genetic detecting method (FemtoPath). **Results:** Our results showed that FemtoPath/direct sequencing test identified EGFRM/K-rasW in 10 (43.48%) of the 23 tumors, EGFRV/K-rasM in 2 (8.69%) of the 23 tumors and EGFRM/K-rasM in 11 (47.83%) of the 23 tumors. Patients having both EGFR and K-ras mutations in genotype exon 19 del/K-rasM or L858R/K-rasM or S768N/K-rasM or V769M/K-rasM were separately four, seven, one and one in occurrence. Other rare EGFR mutations were also detected: three M766I, one T790A, one A767T, one Q791I, one S720F, one A767V, and one K860I.

**Conclusions:** Rate of K-ras mutation and combinations of EGFRM/K-rasM mutations highly appear in NSCLC patients of Taiwan. Our results obtained by direct sequencing test could provide more information for understanding the correlation between the reactions of NSCLC patients to TKI therapies and genotype, especially in combinations of mutations.

**Key Words:** Carcinoma, non-small cell; Receptor, epidermal growth factor; Direct DNA sequencing test

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**AP16-PP-0018**

Identification and Expression Analysis of Notch3-Targeting MicroRNAs in Ovarian Serous Carcinomas and Their Clinicopathological Impact

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**Background:** Notch signaling plays an important role in cell differentiation, proliferation, and apoptosis, and we previously reported that high expression of Notch3-targeting microRNAs is associated with the clinicopathological impact of Ovarian Serous Carcinomas.”

**Key Words:** Notch3-targeting microRNA; Ovarian Serous Carcinoma; Clinicopathological impact

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**AP16-PP-0019**

Identification and Expression Analysis of Notch3-Targeting MicroRNAs in Ovarian Serous Carcinomas and Their Clinicopathological Impact

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**Background:** Notch signaling plays an important role in cell differentiation, proliferation, and apoptosis, and we previously reported that high expression of Notch3-targeting microRNAs is associated with the clinicopathological impact of Ovarian Serous Carcinomas.”

**Key Words:** Notch3-targeting microRNA; Ovarian Serous Carcinoma; Clinicopathological impact
expression of Notch 3 was associated with poor prognosis and chemoresistance in ovarian serous carcinomas. **Methods:** To identify microRNAs regulating Notch 3 signaling, we searched for microRNAs targeting Notch 3, using microRNA web database (Mirbase, Pic Tar, and Target scan). We examined the expression of candidate microRNAs in ovarian cancer cell lines and 35 human ovarian cancer tissue (22 chemosensitive and 13 chemoresistant cancers) using real-time reverse transcription polymerase chain reaction, and compared their expression between chemosensitive and chemoresistant groups. We also analyzed the correlation of their expression and clinicopathological parameters to assess their clinical impact. **Results:** The candidate microRNAs targeting Notch 3 were miR-136, miR-150, miR-203, miR-512, miR-591, miR-1284, miR-4419a, miR-4495, miR-4510, and miR-4689. Among these microRNAs, miR-136, miR-150, and miR-4510 were downregulated in chemoresistant ovarian cancer cell lines and cancer tissue compared with chemosensitive cell lines and cancer tissues. The low expression (<0.5-fold compared to that of normal epithelial cells) of miR-136 correlated with high stage and lymph node metastasis, and low expression of miR-4510 correlated with lymph node metastasis. The patients with low expression of miR-136 showed significantly worse survival. **Conclusions:** Notch signaling-related microRNAs, miR-136 and miR-4510 were associated with chemoresistance and poor prognostic factors in ovarian serous carcinomas.

**Key Words:** Ovarian neoplasms; Notch signaling; MicroRNA; Chemoresistance

### PTEN Inactivation Is a Late Event and a Poor Prognostic Factor in Clear Cell Renal Cell Carcinoma

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**Background:** Clear cell renal cell carcinoma (ccRCC) is the most common type of renal cell carcinoma. However it is not well known about the molecular mechanism of ccRCC progression. We investigated molecular alterations associated with in ccRCC progression. **Methods:** We investigated mutations occurring in ccRCC with larger tumor size from 424 The Cancer Genome Atlas (TCGA) data for pilot analysis. PTEN mutations were observed in 14 samples only those tumor size were larger than 5 cm. Further analysis for PTEN was done including copy number variation, epigenetic changes, protein expression and prognosis from TCGA data. **Results:** Homozygous deletion (0.9%), heterozygous deletion with mutation (1.9%), heterozygous deletion (19.4%), and mutation (2.2%) were found among 314 samples. Those genomic alterations were more frequently observed in late tumor stage and higher histologic grade. Inactivation of both PTEN alleles was associated with poor prognosis independently of tumor stages and patient age in late stage ccRCC ( hazard ratio, 2.6; 95% confidence interval, 1.1 to 6.2; \( p=0.03 \)). Promoter lesion of PTEN was not hypermethylated. PTEN-targeting miRNAs weakly correlated with protein expression \( (r^2 \leq 8\%) \). **Conclusions:** PTEN inactivation by somatic mutation and deletion is a late event of ccRCC and may be associated with tumor progression. Both PTEN alleles inactivation is an independent prognostic factor. Epigenetic changes including methylation or miRNA seems not to be a contributory factor for PTEN inactivation.

**Key Words:** PTEN protein, human; Carcinoma, renal cell; Prognosis

### Acquired Recurrent Genomic Alteration Links Del(5q) and/or -7/ Del(7q) in the Progression of Myelodysplastic Syndrome and Acute Myeloid Leukemia

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**Background:** Del(5q) and -7/del(7q) are the most common chromosomal abnormalities in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) and are often accompanied each other with poor prognosis. We have evaluated relationship between -5/del(5q) and -7/del(7q) in the background of genomic alteration. **Methods:** Integrated analyses of 26 cases with MDS/AML carrying del(5) and/or -7/del(7q) were performed by using karyotype, fluorescence in situ hybridization and array comparative genomic hybridization and characterized by grouping: group I, cases only with del(5q); group II, cases only with -7/del(7q); and group III, cases with concurrent del(5q) and del(7q). **Results:** The overlapped common deleted region of chromosome 5 from group I and III was 5q31.1-33.1 (130,562,020-150,625,216 bp; hg18) and chromosome 7 from group II and III was 7q31.31-q36.1 (119,547,309-149,033,790 bp; hg 18). Total 249 other copy number alterations (CNAs) from the genome except chromosomes 5 and 7, which were ~78.3% of total CNAs, were observed. **Conclusions:** The group III is a distinctive entity carrying the most numerous other CNAs, recurrent other CNAs, cryptic CNAs (can <5 Mb) and complex CNAs. Gains of MLL and ERG or loss of TP53 were highly associated with del(5q) since they are common in both groups I and III. Whereas the loss of ETV6 shown to be specially associated with group III. Theses CNAs or genes associated with particular group or particular chromosomal abnormality may play secondary role of group specific disease progression and should be further evaluated for their clinical significance and influence on therapeutic approaches in the patient with MDS/AML carrying del(5q) and/or -7/del(7q).

**Key Words:** Myelodysplastic syndrome; Leukemia, myeloid, acute; Chromosome 5; Chromosome 7; Copy number alteration

### Interpretation of Low Level BRAF Mutation by Pyrosequencing

**AP16-PP-0021**
Carcinoma. We performed microRNA array analysis using total RNA extracted from fresh cytological specimens of invasive lung adenocarcinoma (IGBP1+) and minimally invasive adenocarcinoma (IGBP1-) and sought candidate microRNAs that were associated with IGBP1-expression. We then compared the results of microRNA array data with reverse transcription-polymerase chain reaction, and pyrosequencing. As most of the methods are not reliable for the detection of low level mutation, its interpretation could be unstable. Therefore it should be observed carefully, because most of them could be a true mutation.

Key Words: MicroRNAs; Adenocarcinoma; Lung

MicroRNAs Implicated in the Control of Abnormal Immunoglobulin Binding Protein 1 Expression in Lung Adenocarcinoma
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Background: Binding of immunoglobulin binding protein 1 (IGBP1) to PP2Ac has an anti-apoptotic effect. We have already reported that overexpression of IGBP1 occurs during the course of malignant progression in lung adenocarcinoma. However, the molecular mechanism of IGBP1-overexpression is still unclear. Although there are few reports of mutation, hypomethylation, or amplification of IGBP1, down-regulation of miR-34b has been reported to lead to high expression of IGBP1.

Methods: We performed microRNA array analysis using total RNA extracted from fresh cytological specimens of invasive lung adenocarcinoma (IGBP1+) and minimally invasive adenocarcinoma (IGBP1-) and sought candidate microRNAs that were associated with IGBP1-expression. We then compared the results of microRNA array data with microRNAs listed in TargetScan (a microRNA database) that would potentially bind to IGBP1. Also, using reverse transcription-quantitative polymerase chain reaction (PCR), we analyzed the expression levels of these candidate microRNAs in frozen specimens of lung adenocarcinoma. Results: Using microRNA array and TargetScan data, we selected 7 microRNAs considered to be associated with IGBP1 expression, and compared their expression between IGBP1-positive adenocarcinomas and IGBP1-negative normal lung tissue using reverse transcription-polymerase chain reaction. Among these microRNAs, miR-34b, -138, -374a/b, -1909, and -3941 were down-regulated in invasive adenocarcinoma (IGBP1+) relative to normal lung tissue.

Conclusions: We have identified 6 candidate-microRNAs that are thought to control IGBP1 expression. Each microRNA is down-regulated in IGBP1-positive lung adenocarcinoma relative to adjacent normal lung tissue.

Key Words: MicroRNAs; Adenocarcinoma; Lung

Detection of Epidermal Growth Factor Receptor and KRAS Mutation by Pyrosequencing Analysis in Cytologic Samples of Thyroid Carcinoma (PTC) and detected by various methods including direct sequencing, allele-specific melting curve analysis, denaturing high performance liquid chromatography, real-time polymerase chain reaction, and pyrosequencing. As most of the methods are not reliable for the detection of low level mutation, its interpretation could be a changing problem in molecular diagnostic laboratory. Therefore we investigated the significance of the low level Braf mutation by pyrosequencing. Methods: A total of 43 PTC with low level (5-7%) Braf mutation were selected. We interpreted mutation positive over 8% mutation, negative under 5% mutation. DNA was extracted from formalin fixed paraffin embedded tissues, Braf codon 599 and 600 mutations were analyzed twice by pyrosequencing. Results: In the first pyrosequencing, prevalence of Braf mutation was as follows: 23.3% (10/43 cases) showed 7% level mutation, 25.6% (11/43) were 6% level mutation, and 51.1% (22/43) were 5% level mutation. The results of second test were changed in 23.3% (10/43), unchanged in 76.7% (33/43); 7% (3/43) were moved to mutation positive (8-9%) and 16.3% (7/43) were changed to mutation negative (3-4%). Conclusions: Braf low level mutation by pyrosequencing is unstable. Therefore it should be observed carefully, because most of them could be a true mutation.

Key Words: Braf low level mutation; Pyrosequencing; Thyroid cancer, papillary
Non-small Cell Lung Cancer: A Better Detection Tool?

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Background: Epidermal growth factor receptor (EGFR) and KRAS mutations are two of the most common mutations in lung cancer. Screening and detecting these mutations are of issue, and many different methods and tissue samples are currently used to effectively detect these two mutations. In this study, we aimed to evaluate the testing for EGFR and KRAS mutations by pyrosequencing method, and compared the yield of cytology versus histology specimens.

Methods: We retrospectively reviewed EGFR and KRAS mutation results of 399 (patients with EGFR mutation test) and 323 patients (patients with KRAS mutation test) diagnosed as lung cancer in Konkuk University Medical Center, Seoul, Korea, from January 2006 to September 2012. Medical records were reviewed including patient age, gender, history of smoking, pathologic diagnosis, and diagnostic methods. The mutation tests were performed by pyrosequencing method. We compared the detection rate of EGFR and KRAS tests in cytology, biopsy, and resection specimens.

Results: EGFR and KRAS mutations were detected in 29.82% and 8.66%, and the positive mutation results of EGFR and KRAS were mutually exclusive. The detection rate of EGFR mutation in cytology was higher than non-cytology (biopsy or resection) materials (cytology, 48.48%; non-cytology, 26.12%), and the detection rate of KRAS mutation in cytology specimens was comparable to non-cytology specimens (cytology, 8.34%; non-cytology, 8.73%).

Conclusions: We suggest in this study that cytology specimens are good alternatives that can readily substitute tissue samples for testing both EGFR and KRAS mutations. Moreover, pyrosequencing method is highly sensitive in detecting EGFR and KRAS mutations in lung cancer patients.

Key Words: Genes, erbB-1; KRAS; Pyrosequencing; Cytology; Mutation test