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Abstracts

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Abstracts

Association of Apolipoprotein E Genotypes with Disease Progression in Hepatitis B Virus Infected Patients

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Apolipoprotein E (ApoE) plays an important role in regulating the lipid and lipoprotein metabolism. We investigated whether the ApoE genotype could determine the disease outcome in hepatitis B virus (HBV) infected individuals to verify the association with the occurrence of hepatocellular carcinoma (HCC) in patients with chronic liver diseases of various etiologies. This hospital based case-control study enrolled 183 subjects (47 healthy controls, 50 HBV-originated liver cirrhosis, and 86 HCC). ApoE genotypes were determined with an ApoE genotyping kit, using the PCR method. Serum ApoE levels were measured with an ELISA kit. We identified that the ApoE genotype was associated with the progression to liver cirrhosis in chronic HBV carriers. Being an ApoE4 carrier was associated with a lower probability of developing liver cirrhosis. No influence of ApoE genotypes on the susceptibility to the occurrence of HCC was found. The serum ApoE measurements revealed a significantly higher level of ApoE in patients with liver cirrhosis than those in the healthy controls, but we observed no significant difference in the serum ApoE levels, with regard to the ApoE genotype. This study indicates that ApoE genotypes may be a part of the genetic variation underlying the susceptibility of individuals to disease progression of chronic HBV infection.

Factors Related to Deterioration of Liver Function after Radiofrequency for Hepatocellular Carcinoma: A 48-month Prospective Follow-up

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Background: Maintaining residual liver function is an important prognostic factor in patients treated with RFA. We aimed to evaluate changes in liver function parameters and risk factors 48 months after RFA therapy in patients with hepatocellular carcinoma (HCC).

Methods: We reviewed 55 patients with HCC who had undergone RFA as initial therapy and showed no recurrence in 48 months. Serial changes in serum total bilirubin, albumin, prothrombin time and Child-Pugh score were evaluated before and after RFA. Patients with chronic hepatitis B received oral nucleoside therapy. Aggravation of liver function after RFA therapy was defined as an increase in the Child-Pugh score by 2 or more at 48 months after RFA therapy. A logistic regression analysis was used to determine the risk factors for aggravation of liver function after RFA therapy.

Results: There was a single tumor in 44 patients and multiple tumors in 11 patients. Single ablation was performed in 18 patients with multiple ablations in 37 patients. The mean ablation duration was 21.8 ± 2.3 minutes. There were 31 patients in Child class A, 17 in child class B and 3 in Child class C. Serum albumin levels showed a significant decrease from before (3.52 ± 0.67 g/dL) to 48 months after RFA therapy (3.01 ± 0.72 g/dL; $P=0.031$). The Child-Pugh score significantly increased from before (6.24 ± 1.47) to 48 months after RFA therapy (7.68 ± 2.07 ; $P=0.026$). A stepwise multivariate analysis showed pre-RFA thrombocytopenia ($<75,000/\text{mm}^3$) as a significant risk factor for long-term aggravation of liver function after RFA. However, there were no cases of bleeding as a complication of RFA in patients with thrombocytopenia.

Conclusions: Liver function parameters, particularly the serum albumin level, gradually decreased in HCC patients over the course of 48 months after RFA therapy. The presence of pre-RFA thrombocytopenia represents a major risk factor for the aggravation of liver function, but not for bleeding after RFA therapy.

Epidemiology, Prognosis and Clinical Features of Hepatocellular Carcinoma in Mongolia

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Background and Aim: Hepatocellular carcinoma (HCC) is the most common cancer in Mongolia. We aimed to investigate the clinical features, therapeutic modalities, overall survival, and prognostic factors for Mongolian patients with HCC.

Method: One hundred ninety-five patients with HCC were consecutively enrolled in our study.

Results: The mean age was 61.7 years. The most common etiology for HCC was HCV infection (n=89, 45.6%), followed by HBV infection (n=67, 34.4%). The mean tumor diameter was 6.0 ± 2.6 cm. Only 29 (14.9%) patients had a single lesion, while 39 (20.2%) had >3 lesions. Extrahepatic metastasis to lung (n=23), bone (n=10), and lymph node (n=3) was detected in 36 (18.5%) patients. Most patients had advanced HCC; 88 (45.1%) in stage III and 57 (29.2%) in stage IV. Surgical resection was performed in 27 (13.8%) patients, RFA in 23 (11.8%), and TACE in 107 (54.9%). When all the patients were categorized as 'treated' (n=156) and 'not treated' (n=39), the 3 year survival was significantly lower in the 'not treated' group than in the 'treated' group (11% vs. 0%, P<0.001). Tumor diameter (<3 cm vs. ≥3 cm), extrahepatic metastasis, TNM stage (I/II vs. III/IV), and treatment (or supportive care) were selected as independent predictors for survival.

Conclusions: A high proportion of patients with HCC in Mongolia is diagnosed at an advanced stage and survival of these patients is lower compared to other countries. A surveillance system and referral policy for high risk groups should be urgently established and implemented in Mongolia.

Feasibility and Efficacy of Stereotactic Ablative Radiotherapy for Barcelona Clinical Liver Cancer-C Hepatocellular Carcinoma

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Aim: To demonstrate the feasibility and efficacy of stereotactic ablative radiotherapy (SABR) for Barcelona Clinical Liver Cancer (BCLC)-C hepatocellular carcinoma (HCC).

Methods: We retrospectively reviewed the clinical records of 35 patients treated with SABR for BCLC-C HCC between 2003 and 2011. According to pre-SABR radiology, vascular invasion

(portal vein or inferior vena cava tumor thrombosis) was diagnosed in 24 patients (69%) and extrahepatic spread in 11 patients (31%). There were 32 patients (91%) with Child-Pugh classification (CP)-A and 3 patients (9%) with CP-B. SABR was targeted to vascular invasion or extrahepatic spread with (curative field, n = 18, 51%) or without primary HCC (palliative field, n = 17, 49%). The median SABR dose was 45 Gy (range, 30–60 Gy) in 3–5 fractionations. Twenty patients (57%) received further treatment after SABR.

Results: The median follow-up from SABR start dates was 14 months (range, 1–44 months) with a median survival of 14 months. The 1-and 2-year overall survival (OS) rates were 52% and 29%, respectively. On multivariate analysis, CP, tumor response to SABR and a treatable lesion (treated with biologically effective dose (BED) ≥ 80 Gy₁₀ and curative field) were statistically significant factors associated with OS (P < 0.05). Severe toxicity above grade 3 was observed in 5 patients (14%): hepatotoxicity in 1 patient; gastrointestinal toxicity in 3 patients; and myelopathy in 1 patient.

Conclusions: SABR for BCLC-C HCC showed favorable survival rates but the treatment-related toxicity was moderate. This result might suggest that SABR is a treatment option for selected patients having a treatable lesion with BED ≥ 80 Gy₁₀ and curative field among BCLC-C HCC. However, SABR is not recommended for patients with CP-B, considering the poor prognosis and moderate treatment-related toxicity.

Engaging Stakeholders in the Development and Implementation of Comprehensive Liver Cancer Control in China

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Background: Primary liver cancer is the 2nd leading cause of cancer deaths in China with 360,000 incident cases and 350,000 deaths annually. A complex etiology and diversity of disciplines preventing and treating liver cancer imply that a comprehensive control plan is needed. We sought to engage stakeholders in China to elicit their views about current needs and priorities for comprehensive liver cancer control, and their motivation to implement such a plan.

Methods: Clinical, policy and advocacy stakeholders in China completed a survey designed after in-depth qualitative work and piloting. Each respondent completed a needs assessment task (rating and ranking China's performance on 10 indicators), a joint analysis (choosing between systematically identified pairs of 11 liver cancer strategies) and a task measuring stakeholders'

motivation to implement comprehensive liver cancer control. Data were analyzed using univariate and multivariate statistical analyses.

Results: 20 stakeholders completed the survey (response rate=80%). Respondents were predominately focused on hepatocellular carcinoma (70%) and had a national profile (55%). The assessment/management of lifestyle risk factors ($p=0.001$), public awareness and advocacy ($p=0.017$) and national statistics ($p=0.045$) were seen as having the greatest need by respondents. Highest priority was given to the development of national standards and guidelines ($p<0.001$), improved access to recommended treatments ($p<0.001$) and continuous surveillance of at-risk populations ($p=0.001$). Respondents were highly motivated to implement comprehensive liver cancer control plans ($p=0.001$).

Conclusions: Our study demonstrated the value of engaging stakeholders in the formation and implementation of comprehensive cancer control plans. It also demonstrated that a country's needs are not always reflected in its priorities (e.g. advocacy and national statistics were identified as needs but not priorities). This study was limited by a small sample size, but future research can be implemented to test the generalizability of these results and to explore variations across China.

Tigatuzumab, an Anti-human Death Receptor 5 Antibody, Synergizes Sorafenib in Hepatocellular Carcinoma through SHP-1-dependent STAT3 Inactivation

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Background: The recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-cancer therapy. However, most hepatocellular carcinoma (HCC) cells show resistance to TRAIL-induced apoptosis. Tigatuzumab, formerly CS-1008, is a humanized anti-human death receptor 5 (DR5) agonistic antibody. Sorafenib, a multiple tyrosine kinase inhibitor, is the only approved drug for HCC. In this study, we demonstrated that the combination of sorafenib and tigatuzumab overcame the resistance and restored the sensitivity of HCC cells to tigatuzumab.

Methods: HCC cell lines (PLC5, Huh-7, and Hep3B) were treated with tigatuzumab and/or sorafenib and analyzed in terms of apoptosis and signal transductions.

Results: HCC cells, including PLC5, Huh-7, and Hep3B, showed significant resistance to tigatuzumab-induced apoptosis (up to 1000 ng/ml). The combination of sorafenib (starting at 5 μ M) and tigatuzumab restored the sensitivity of HCC cells to tig-

atuzumab-induced apoptosis. Our data showed that signal transducers and activators of transcription 3 (STAT3) played a significant role in mediating tigatuzumab sensitization of sorafenib. Sorafenib down-regulated phospho-STAT3 (Tyr 705) and subsequently reduced the protein levels of STAT3-regulated proteins, Mcl-1, survivin and cyclin D1, in tigatuzumab-treated cells. Knockdown of STAT3 by RNA-interference overcame apoptotic resistance to tigatuzumab in HCC cells, and ectopic expression of STAT3 in HCC cells abolished the sensitizing effect of sorafenib on tigatuzumab. Importantly, the inhibition of SHP-1 by adding a specific SHP-1 inhibitor reduced the effects of sorafenib and tigatuzumab on p-STAT3 and apoptosis whereas co-treatment with tigatuzumab and sorafenib increased the activity of SHP-1, suggesting that SHP-1 mediated the combined effect of tigatuzumab and sorafenib. Moreover, the combination of tigatuzumab and sorafenib inhibited HCC xenograft tumor growth in vivo.

Conclusions: tigatuzumab synergizes HCC cells to sorafenib-induced apoptosis at clinical achievable concentrations, and this effect is mediated through SHP-1 dependent STAT3 inhibition. This study is supported by grants, NSC 100-3112-B-002-013, and NSC 100-2325-B-002-036, and tigatuzumab was kindly provided by Daiichi Sankyo Co. (Tokyo, Japan).

FOXP3 Gene Polymorphism Is Associated with Hepatitis B-related Hepatocellular Carcinoma in China

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Background: Previous evidence has shown that the FOXP3 gene was involved in the pathogenesis of several tumors, but the correlation between single nucleotide polymorphisms (SNPs) in the FOXP3 gene and the susceptibility of hepatitis B-related hepatocellular carcinoma (HCC) remains unclear.

Methods: Two SNPs in the FOXP3 gene, rs2280883 and rs3761549, in 392 patients with HCC, 344 patients with chronic hepatitis B (CHB) and 372 matched healthy controls were analysed with Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry.

Results: At rs3761549, the frequency of the C allele was significantly higher ($P = 0.03$) in HCC patients than in CHB patients and healthy controls. Compared with healthy controls, patients with HCC had higher frequencies of the TT genotype (79.6%) at rs2280883 or the CC genotype (77.6%) at rs3761549 of the FOXP3 gene, while patients with CHB also had higher frequencies of the TT genotype (74.1%) at rs2280883 or the CC genotype (74.6%) at rs3761549. There were no significant differences in

the distribution of FOXP3 genotypes between CHB donors and HCC donors. Stratified analysis showed that the CC genotype of rs3761549 was significantly associated with a high incidence of portal vein tumour thrombus ($P = 0.02$), and the rs3761549 TT/CT genotype was significantly associated with a high rate of tumour recurrence in HCC patients ($P = 0.001$).

Conclusions: These results suggested that the FOXP3 gene polymorphisms at rs2280883 and rs3761549 might be associated with increased susceptibility to hepatitis B-related HCC. At rs3761549, the CC genotype and TT/CT genotype were associated with a high incidence of portal vein tumour thrombus and tumour recurrence, respectively.

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Synchronous Development of Intrahepatic Cholangiocarcinoma and Hepatocellular Carcinoma in Different Sites of The Liver with Chronic B-viral Hepatitis

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Background: The synchronous development of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) has rarely been reported, when it is in the form of double primary cancer. In a literature review, there have only been 35 reported cases of synchronous HCC and ICC, and most of these tumors developed from livers with hepatitis C infections. We present herein on the synchronous development of HCC and ICC in different sites of the liver with chronic B-viral hepatitis.

Case: Two patients who had been followed with chronic B-viral hepatitis were referred for a hepatic mass. One patient (Case 1, a 66-year-old woman) had a 6.4 cm multinodular hepatic mass in the left lobe and a small nodule in the right lobe. Another patient (Case 2, a 68-year-old woman) had a 4.3 cm hypervascular mass in the right lobe and 1.1 cm sized nodule in the left lobe. Radiological examination including CT and MRI was performed and pre-operative diagnosis of both cases was HCC with a metastatic nodule. Thereafter, patients underwent curative resection of the tumors. However, unexpectedly, pathological examination of the surgical specimens revealed that ICC and HCC existed independently in the other side of the same liver in both patients.

Conclusions: These cases not only suggested that double cancer of HCC and ICC could arise from liver with HBV infection, but also emphasize the importance of an intensive diagnostic approach when separate tumor exist in the same liver.

Analysis of Clinical Parameters as A Predictor of The Outcome in Sorafenib-treated Patients with Advanced Hepatocellular Carcinoma

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Background: Sorafenib is an orally active multikinase inhibitor that is approved for the treatment of advanced hepatocellular carcinoma (HCC). However, clinical parameters that may predict the treatment outcomes in sorafenib-treated advanced HCC patients remain unknown.

Methods: A total of 93 advanced HCC patients who were treated with sorafenib as the initial treatment modality from January 2007 to September 2011 were retrospectively reviewed. Overall survival was the primary endpoint for the analysis. Various clinical parameters including patient demographics, Child-Pugh score, portal vein thrombosis, lymph node metastasis, positron emission tomography (PET) standardized uptake value (SUV) of the most hypermetabolic lesion, and adverse effects to sorafenib were analyzed. Univariate and multivariate analyses were carried out to identify clinical parameters as a predictor of the effect of sorafenib.

Results: There were 82 males and 11 females included in the study, with a mean age of 54 years. The overall survival was 3.4 months (95% CI: 2.5–4.3). 92 patients were Child-Pugh class A or B and 1 Child-Pugh class C patient was included. The BCLC staging of the patients was advanced in 92 patients and terminal in 1 patient. Chronic hepatitis B was the predominant cause of HCC (79.5%). Noted adverse effects were hand-foot syndrome, diarrhea, fatigue, abdominal pain, nausea and stomatitis. The univariate analysis showed that the presence of adverse effects such as hand-foot syndrome ($n=22$) and diarrhea ($n=19$) was associated with a longer overall survival duration (6.7 months vs 2.5 months, $p=0.014$ and 6.6 months vs 2.7 months $p=0.044$, respectively). The presence of hand-food syndrome predicted a better overall survival in the multivariate analysis ($p=0.001$).

Conclusions: Advanced HCC patients treated with sorafenib who experienced hand-foot syndrome and diarrhea showed better overall survival than patients without these side effects. These side effects may be used as clinical parameters for the prediction of sorafenib response in patients with HCC.

Imaging Diagnosis of Very Small Hepatocellular Carcinoma in Gd-EOB-DTPA-enhanced and Diffusion-weighted MRI

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Background: It is necessary to refine the new diagnostic criteria for hepatocellular carcinoma (HCC) using the most recent MRI techniques beyond the conventional dynamic imaging techniques that rely on only the tumor vascular pattern. We evaluate herein the usefulness of MRI criteria with Gd-EOB-DTPA-enhanced MRI and diffusion-weighted imaging (DWI) for the diagnosis of very small (≤ 2.0 cm) HCCs.

Methods: Fifty-four patients with 51 HCCs and 12 dysplastic nodules (DNs) (≤ 2.0 cm) underwent Gd-EOB-DTPA-enhanced MRI and DWI. MRI criteria for HCC were as follows: (1) arterial hyperenhancement and hypointensity on the hepatobiliary phase (HBP) with/without hyperintensity on DWI; (2) hypovascular nodule with hyperintensity on DWI; (3) arterial hyperenhancement and either iso- or hyperintensity on HBP, with hyperintensity on DWI and (4) hyperintensity only on DWI. According to the MRI criteria, MRI findings for HCCs and DN were independently classified by two reviewers.

Results: Among 51 HCCs, 42 HCCs (82%) showed arterial hyperenhancement and hypointensity on HBP, and hyperintensity on DWI. For each observer, 50 (98%) and 49 HCCs (96%) were discernible with MR criteria, respectively. For both observers, a tiny HCC (0.6 cm in diameter) was overlooked, as it showed subtle hyperintensity only on DWI, but was not seen on other images. Out of 12 DN, 10 nodules were seen as hypointense only on HBP, but were not depicted on other images, including arterial phase and DWI. However, two remaining high-grade DN were seen as either iso- or hypointense on arterial phase and hypointense on HBP, with hyperintensity on DWI.

Conclusion: We could reliably diagnose very small HCCs with the HCC imaging criteria based on combined Gd-EOB-DTPA-enhanced MRI and DWI.

Viable Tumors with Dynamic Contrast-enhanced Magnetic Resonance Imaging in Spontaneous Necrosis of Liver VX2 Tumors in Rabbits

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Background: Dynamic contrast-enhanced (DCE)-MRI has been shown to be clinically feasible and useful in predicting and measuring the response to therapy in many tumors. However, few investigations of DCE-MRI on the evaluation of viable tumors in

liver tumors have been described. The purpose of this study was to compare the accuracy of DCE-MRI with pathology for predicting viable tumors in hepatic VX2 tumors with spontaneous necrosis in rabbits.

Methods: Ten rabbits with ten experimentally induced VX2 tumors (one tumor from each rabbit) in the liver underwent DCE-MRI and T2 weighted imaging (WI). All rabbits were sacrificed immediately after DCE-MRI and extracted livers were dissected in the coronal plane with slices similar to those of MR imaging with 4 mm intervals. After hematoxylin-eosin (H-E) staining, complete and partial necrotic areas of all tumors were evaluated pathologically. The necrotic area of tumors on the T1 map including the K^{trans} , area under the curve (AUC) and signal intensity (SI) during one phase of DCE-MRI were compared with the pathological findings. Two radiologists recorded the grade of concordance between MR images and the pathology. Subjective grading was assessed with the Wilcoxon sign rank test. Kappa statistics were used to assess the interobserver agreement.

Results: In subjective grading, the AUC had the mean highest score and SI during one phase of DCE-MRI scored second highest in both complete and partial necrosis evaluation. The AUC showed significantly superior concordance with the pathology to the K^{trans} and SI during one phase of DCE-MRI ($p < 0.05$). SI during one phase of DCE-MRI showed significantly superior concordance to the K^{trans} ($p < 0.05$). The kappa values among the two radiologists showed moderate agreement.

Conclusion: DCE-MRI could considerably reflect a viable and necrotic tumor in concord with the pathology. In particular, the AUC could be helpful for prediction of viable tumors in spontaneous necrosis of liver VX2 tumors in rabbits.

The Optimal Indication of Sorafenib Treatment in Patients with Advanced-Stage Hepatocellular Carcinoma

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Background: Although sorafenib treatment is the current standard of care in patients with hepatocellular carcinoma (HCC) at an advanced-stage (BCLC C stage), it may be insufficient to achieve satisfactory results in some patients. We investigated clinical factors predictive of survival end-point following sorafenib treatment in such patients.

Methods: This study included 191 patients with advanced-stage HCC who received sorafenib monotherapy as initial or rescue therapy during at least 5 weeks. We analyzed the predictors

of overall survival (OS) in these patients using the Kaplan-Meier method and Cox regression, and then clarified a selected group of patients with the greatest survival benefit for sorafenib treatment.

Results: Of 191 patients, 43 (22.5%) were initially treated with sorafenib, and 55 (28.8%) had Child-Pugh class B liver disease. There were 94 patients (49.2%) with vascular invasion, 20 (89.5%) with extrahepatic spread, and 74 (38.7%) with both. The median treatment duration was 16 weeks (range, 5.3–113.4), sorafenib was initiated at a low starting dose of 200 mg bid in 60 patients (31.4%), and 66 (34.6%) required dose modification. With a median follow-up of 7.0 months (range, 1.4–43.6), the median OS was 9.6 months (95% CI, 7.9–11.4). In univariate and subsequent multivariate analyses, Child-Pugh class B, bilobar involvement of tumors, vascular invasion, and sorafenib as rescue therapy were independent predictors affecting unfavorable OS (HR 2.10, 1.68, 1.71, and 2.02, respectively; $P < 0.05$ for all). The median OS periods were 16.6 months in patients without any identified risk factors, which were significantly greater than 7.6 months in those with ≥ 1 risk factors ($P = 0.001$).

Conclusion: Better liver function, limited tumor extent, no vascular invasion, and first-line treatment were strongly associated with slower progression and longer survival after sorafenib monotherapy in patients with advanced-stage HCC. A favorable survival outcome following sorafenib treatment could be clinically predicted in selected patients having all the good prognostic factors.

Bone Metastases from Hepatocellular Carcinoma: Comparison of ^{18}F -FDG PET/CT with $^{99\text{m}}\text{Tc}$ -HDP Bone Scintigraphy and Correlation with Clinical Features

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Background: The purpose of this retrospective study was to elucidate the clinical features of bone metastases from HCC and compare the sensitivities of ^{18}F -fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) and bone scintigraphy (BS).

Materials and Methods: Between January 2005 and October 2010, 67 consecutive patients who had undergone both PET/CT and BS within a 3-month interval were evaluated.

Results: Bone metastases were most frequently found in the pelvis (20%), followed by the lumbar spine (14%), and long bones (13%). PET/CT was significantly more sensitive than BS in region-based analysis with 273 confirmed bone metastases

(96.7% vs. 52.7%, $p < 0.001$) and in patient-based analysis (99% vs. 85%, $p = 0.042$). The median survival period was 5 months (range: 0.4–18 months). A univariate analysis revealed that poor prognostic factors included age (< 60 years), multiple bone metastases, lymph node metastasis, high serum alpha-fetoprotein (AFP) (≥ 400 IU/ml), Child-Pugh class B, and high SUV_{max} (> 5.0). Both high AFP (≥ 400) and high SUV_{max} (> 5.0) associated with primary HCC were poor prognostic factors. Large metabolic volume ($\geq 200 \text{ cm}^3$) of bone metastasis was another poor prognostic factor. A Cox-regression analysis showed that high AFP was the only poor prognostic factor with significance.

Conclusions: PET/CT was more sensitive than BS both on patient-based and region-based analyses, and offered additional information on survival. PET/CT can be helpful in early diagnosis and opportune treatment of bone metastasis from HCC.

Diagnostic Accuracy of Gadoteric Acid Enhanced Magnetic Resonance Imaging in Predicting the Appropriateness of a Transplant Recipient Based on the Milan Criteria

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Background: To evaluate and compare the diagnostic accuracy of gadoteric acid enhanced magnetic resonance imaging (MRI) in predicting the appropriateness of a transplant recipient with hepatocellular carcinoma (HCC) based on the Milan Criteria.

Methods: Forty five patients who underwent liver transplantation for liver cirrhosis accompanied by viable hepatocellular carcinoma between January 1, 2009, and December 31, 2011, were retrospectively reviewed. Two radiologists reviewed in consensus the pre-operative gadoteric acid enhanced MRI findings for the presence and size of viable HCC nodules, vascular invasion and eligibility for transplantation based on the Milan criteria. They made a diagnosis of HCC based on the AASLD criteria (arterial enhancement and wash out) or new criteria, which consist of three of five imaging findings favoring HCC (arterial enhancement, wash out, high signal intensities on T2 weighted image, high signal intensities on diffusion weighted images, and low signal intensity on hepatobiliary phase images). The post-operative pathologic findings were correlated with the pre-operative interpretation. The diagnostic performance of the two imaging sets was compared using McNemar's test.

Results: Among 45 enrolled patients, 18 recipients were found to have failed to meet the Milan criteria according to the post-operative pathologic examination. The sensitivities of AASLD and the new criteria for predicting the recipient classified as beyond the Milan criteria were 16.7% and 50.0%, respectively. Specificities and accuracies of both image sets were 96.3% and 96.3%, and 64.4% and 77.8%, respectively. Sensitivities and accu-

racies showed a significant difference ($p=0.0412$, 0.0233 , respectively). The most common cause of false negative was tumors less than 1 cm.

Conclusion: New criteria for diagnosing HCC from gadoxetic acid enhanced MRI showed significantly better diagnostic accuracies than the AASLD criteria. Gadaxetic acid enhanced MRI showed very high specificity and moderate sensitivity for assessing the appropriateness of a transplant recipient based on the Milan criteria.

Reduced Failure Rate of Hepatocellular Carcinoma Surveillance by Adopting Computed Tomography or Magnetic Resonance Imaging as an Alternative Screening Method in Patients with Macronodular Cirrhosis

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Background & Aims: Initial presentation of hepatocellular carcinoma (HCC) at an advanced stage in patients under a regular surveillance program is a devastating problem. We assessed the prevalence and clinical factors associated with this surveillance failure.

Methods: A total of 304 patients who were diagnosed as having HCC under a regular surveillance program were retrospectively reviewed. Surveillance failure was defined when the tumor was diagnosed at beyond the Milan criteria. The surveillance programs consisted of radiological tests [ultrasonography (US) for the US-only surveillance (US-S) group ($n = 48$, 15.8%); US with computed tomography or magnetic resonance imaging for the alternative surveillance (Alt-S) group ($n = 256$, 84.2%)] and measurement of serum alpha-fetoprotein at 6 month intervals. We evaluated the factors associated with surveillance failure.

Results: Surveillance failure was noted in 18 of 304 patients (5.9%). Macronodular cirrhosis (MC) (yes vs. no: 10.3% vs. 3.2%; odds ratio (OR) 4.22; 95% confidence interval (CI): 1.28 – 13.90), US-S (yes vs. no: 14.6% vs. 4.3%; OR 3.59; 95% CI: 1.04 – 12.47), and infiltrative tumor type (yes vs. no: 57.1% vs. 3.4%; OR 35.38; 95% confidence interval CI: 9.09 – 138.33) were independent factors for surveillance failure. Based on the two baseline factors, the surveillance failure rates were 35.7%, 6.8%, 5.9% and 2.6% for MC (+)/US-S, MC (+)/Alt-S, MC (-)/US-S, and MC (-)/Alt-S, respectively ($p < 0.001$).

Conclusions: The HCC surveillance failure rate was significantly higher for patients with MC who underwent US-S. Surveillance failure risk could be reduced by Alt-S in these patients.

Suppression of Tumor Growth and Invasion through Inhibition of HIF Dimerization

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Objectives: HIF-1 β (ARNT) is a constructed dimerization of HIF-1 α , which is involved in various aspects of cancer biology, including proliferation and survival under hypoxic conditions. This in vitro study investigated the mechanism by which small interference HIF-1 β (siHIF-1 β) inhibits HIF-1 α dimerization, leading to suppression of tumor cell growth.

Method: Three HCC cell lines (HepG2, Huh 7 and Hep3B) were transfected with HIF-1 β -coding siRNA and cultured under hypoxic conditions (1% O₂, 24 hours). Following transfection, the expression levels of HIF-1 β , HIF-1 α , and growth factors were examined with immunoblotting. Additionally, tumor growth was measured with an MTT assay and tumor activity was measured with TUNEL, tumor invasion and migration assays.

Results: The inhibition of HIF-1 β by siHIF-1 β led to suppression of tumor cell growth through inhibition of HIF dimerization under hypoxic conditions. This effect was increased and maximized at 48 hrs after treatment of HIF-1 β coding siRNA. Inhibition of HIF dimerization through treatment with siHIF-1 β also regulated the expression of tumor growth related factors (VEGF, EGF and HGF). Inhibition of HIF-dimerization precluded tumor invasion and migration in a variety of HCC cell lines.

Conclusions: siHIF-1 β -mediated inhibition of HIF dimerization may lead to anti-tumor effects in the hypoxic tumor environment.

Combined TACE and RFA Treatment of HCC Lesions in Contact with Blood Vessels: Local Recurrence Due to Heat Sink Effect

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Purpose: To evaluate the efficacy and safety of combined TACE and RFA treatments in patients with HCCs located near major blood vessels of the liver. The adverse effect of significant perfusion from vessels adjacent to hepatic lesions, as a cause of incomplete coagulation necrosis by RFA due to heat-sink effect,

has been established from early on, however data-evidence of recurrence of these HCC lesions seems to be lacking.

Materials and Methods: We have retrospectively reviewed the imaging studies of patients with HCC who received combined TACE and RFA at our institute from September 2008 to April 2011. A total of 24 HCC lesions that were in close contact (<3 mm) with major blood vessels (>3 mm) was investigated. Imaging review included each patient's initial dynamic liver CT or MRI, followed by CTHA/CTAP and fluoroscopic images obtained during TACE and US images obtained during the RFA procedure, as well as follow up imaging studies. All lesions were initially treated with TACE using an infusion of adriamycin and lipiodol (gelfoam in some cases), and with RFA immediately after TACE using the cool-tip needle type.

Results: The mean diameter of HCC lesions was 2.3 cm, and the median diameter of blood vessels adjacent to these lesions was 6.3 mm, including the hepatic vein in 5 cases, portal vein in 16 cases, IVC in 1 case, and in 2 cases the lesions were in contact with both hepatic and portal veins. The local recurrence rate related to the heat-sink effect for these lesions was 8.3% (2 out of 24) at 1 year. The median follow up period of cases in which the investigated lesion under risk of heat-sink effect did not show recurrence (22 cases) was 29 months. The 2 recurrent cases were observed at 6 and 9 months respectively from combined therapy and were successfully re-treated. No major complications have been observed after the treatments.

Conclusions: It is both safe and efficient to perform combined TACE and RFA treatments on HCC lesions that are under high-risk from the heat-sink effect.

Prognostic Signature Determined by Tumor Stromal Responses in Human Hepatocellular Carcinoma

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Background: The tumor stroma is a source of many potential new tumor biomarkers, however, little is known regarding how changes in stromal gene expression affect hepatocellular carcinoma (HCC) progression. The aim of this study was to elucidate prognostic information based on stromal responses in HCC.

Methods: We analyzed the expression of 28 stroma-related genes using quantitative RT-PCR in 122 HCC samples, and related the results to the patient prognosis. The identified gene classifier was further validated in an independent set of 172 HCC samples. The densities of various tumor stromal cells were analyzed with immunostaining.

Results: Hierarchical clustering based on expression of the 28 genes, or the 6-Th1 cell markers (TBX21, CD3Z, IFNG, IRF1, GZMB and GNLY), can classify HCC patients into prognostically different subgroups. We further identified a five-gene classifier (pro-

tective genes: IRF1, GZMB and risk genes: CRTH2, VEGF, MMP7) as a significant independent prognosticator for recurrence (HR, 4.80; 95% CI, 2.31–9.96; $P = 2.6 \times 10^{-5}$) based on the multivariate analyses. Importantly, the classifier was further validated in an independent set of 172 HCC samples (HR, 2.21; 95% CI, 1.20–3.00; $P = 0.002$). The predictive ability of the classifier, as measured by the AUC (0.713 and 0.613, for original and validation cohorts respectively), was comparable to those of vascular invasion, and the BCLC and TNM stages. Further, immunohistochemistry suggested that protein expression of the five genes mainly presented in the tumor stroma, in particular tumor-infiltrating inflammatory cells, and tumor cells as well. Comparing the results of immunostaining and gene expression data showed the significant association of the gene classifier with the amount of immune/inflammatory infiltration in both cohorts.

Conclusions: The signature reveals the strong prognostic capacity of immune responses, angiogenic activity and extracellular matrix remodeling, highlighting the importance of stromal biology in HCC progression. Contained in this novel predictor may be targets suitable for new therapeutic interventions, or it may be used as independent prognosticator.

Improving Surgical Outcomes after Resection for Hepatocellular Carcinoma over a 10-year Period

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Introduction: Hepatic resection has been considered the treatment of choice for hepatocellular carcinoma (HCC) in patients with preserved liver function. Some studies have reported that long-term outcomes as well as perioperative outcomes have improved over the past two decades. Therefore, we attempted to demonstrate whether there has been improvement in the surgical outcomes of curative resection of HCC from a single center experience of hepatic resection for HCC over a 10-year period.

Methods: From January 1996 to December 2007, 610 patients underwent curative resection for HCC at Yonsei University Health System, Seoul, Korea. First, the long-term outcomes after resection were analyzed and the prognostic factors for disease and overall survival were investigated. Second, the patients were divided according to two time periods: before 2003 (group 1, n=233) and after 2003 (group 2, n=377). Clinicopathologic data and survival results were compared between the two groups.

Results: The 1-, 3-, and 5-year overall survival rates of 610 patients were 90.1%, 74.9% and 64.4%, respectively. After multivariate analysis, perioperative transfusion, multiple tumors, microscopic vascular invasion, Edmondson-Steiner grade (III-IV), albumin ≤ 3.5 g/dL, and indocyanine green retention at 15 minutes > 10% were the independent adverse prognostic factors for over-

all survival. There were no significant differences in perioperative morbidity and mortality rates between the two groups. Although the disease-free survival rates between the two groups were not significantly different, there tended to be improved overall survival rates in the group 2 (5-year overall survival rate- 60.5% in group 1 and 69.9% in group 2, $p=0.098$). Although there were no significant differences in disease-free and overall survival in patients with tumor size ≤ 3 cm, group 2 showed a significantly higher overall survival rate after resection in patients with a tumor size >3 cm. The survival rate after recurrence in patients with >3 cm tumors was also significantly higher in group 2. Group 2 in patients with tumor size >3 cm had a significantly lower proportion of blood transfusion and serum albumin ≤ 3.5 g/dL.

Conclusions: Long-term outcomes after curative resection of HCC were associated with underlying liver function, tumor invasiveness and surgical techniques. The improving survival results in the recent 5 years may be attributable to the proper selection of the surgical candidate, a reduced perioperative transfusion rate as a result of improved surgical techniques and active multimodality treatment of recurrent HCC.

Tumor-associated Neutrophils in Peripheral Blood and Tumor Tissue Foster Immune Privilege via Suppression to T Lymphocytes in Hepatocellular Carcinoma

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Background: Recently, the dual roles of tumor-associated neutrophils (TANs) within the multiple step paradigm of cancer have been recognized. Although there are multiple bodies of evidence that an elevated neutrophils-to-lymphocyte ratio (NLR) predicts a poor prognostic outcome in patients with hepatocellular carcinoma (HCC), the potential molecular mechanisms are still to be identified, which is critical for understanding the immunopathogenesis and for further therapeutical applications.

Methods: Peripheral blood and peritumoral/intratatumoral neutrophils from 110 HCC patients were assessed with flow cytometry. Purified neutrophils were exposed to tumor supernatant from hepatoma cells for phenotypical analysis. For functional analysis, human neutrophils were separated from whole blood and then co-cultured in vitro. Finally, immunohistochemistry was used to analyze the distribution and clinical relevance of neutrophils in 192 HCC patients.

Results: Our results showed that the expression of programmed death ligand 1 (PD-L1), which interacts with PD-1, in-

creased on neutrophils from HCC patients compared with healthy controls and chronic hepatitis B patients. The expression of PD-L1 on neutrophils from peritumoral tissue was much higher than that of intratumoral or non-tumor tissue. The immunohistochemical assay showed that tumor-educated neutrophils were mainly present at the evading edge rather than in the cancer nest or non-tumor tissue. A high infiltration of TANs is also closely correlated with the tissue infiltrated effector T cells.

Conclusions: These data suggest that tumor-associated neutrophils in peripheral blood and tumor tissue of hepatocellular carcinoma with high expression of PD-L1 may suppress the T cell response via cell-cell contact and contribute to the maintenance of immunosuppression and foster immune privilege.

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SY Y Renders Hepatocellular Cancer Sensitive to Oxaliplatin through Inducing CSC Differentiation

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Background: Recent evidence suggests that a certain type of hepatocellular carcinoma (HCC) is hierarchically organized by kinds of cancer cells including a subset of CSCs (Cancer Stem Cells) which have emerged as the driving force of carcinomas. Our team had proved that SY Y (traditional Chinese medicine comprised of 5 herbal extracts) can inhibit molecular changes consistent with EMT in oxaliplatin-treated tumor tissues and cell lines. We investigated herein whether SY Y could induce CSC differentiation and render hepatocellular cancer sensitive to oxaliplatin.

Methods: The human hepatocellular carcinoma cell lines MHCC97H and Hep3B treated with SY Y for 4 weeks were exposed to oxaliplatin in a gradient concentration to identify the chemotherapeutic efficacy. Furthermore, the “stemness” such as the proportion of CSCs, invasion, metastasis, colony forming ability, and tumorigenesis were also examined. The orthotopic nude mouse model of human HCC was developed using MHCC97H and the Chinese herbal extract SY Y was investigated to explore its effect on the volume of surviving tumor, the rate of tumorous metastasis and the proportion of CSCs. The associated molecular changes were evaluated with Western blotting, qRT-PCR and immunohistochemistry.

Results: In vitro, MHCC97H and Hep3B cell lines which were pretreated with SY Y for 4 weeks showed a decreased proportion of CSCs, attenuated migration and invasion ability, reduced tu-

morigenicity and strangely increased oxaliplatin efficacy. In vivo, residual HCCs in orthotopic transplanted recipient nude mice treated with SY Y (4 g/kg) for 5 weeks showed little reduction in tumor volume ($p > 0.05$), whereas a significantly reduced tumor volume ($p < 0.01$) could be seen after oxaliplatin therapy adjuvant with SY Y compared to the oxaliplatin alone. Moreover, pulmonary metastasis ability ($p < 0.01$) and increased life span ($p < 0.01$) could also be seen. Molecular changes consistent with the decreased “stemness” were observed in oxaliplatin and SY Y cotreated tumor tissues and verified with in vitro experiments.

Conclusions: SY Y can renders hepatocellular cancer sensitive to oxaliplatin through inducing hepatocellular cancer stem cell differentiation and reduced the “stemness”

Hepatic Proliferation after Irradiation Injuries

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The current study investigated hepatic proliferation after irradiation injury of the liver.

1. Liver regeneration after partial liver irradiation in rats: The liver has a strong potential for regeneration after physical, biological or chemical injury, but no data have been reported so far on liver regeneration in response to irradiation injury. The present experiment in rats was designed to clarify whether partial liver irradiation could induce and stimulate the unirradiated part of liver to regenerate. The left half of the rat liver was irradiated with a single dose of 25 Gy. Liver tissues from the irradiated and unirradiated liver, and blood sample were collected at different time points after irradiation. Radiation injury was evaluated by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, histopathologic features and trichrome stain. The hepatic regeneration was assessed with serum hepatic growth factor (HGF), mitotic index and proliferation cell nuclear antigen (PCNA) immunohistochemical stain. Expression of transforming growth factor- β 1 (TGF- β 1) as shown with an immunohistochemistry assay was also performed. Results showed that 25 Gy of single dose irradiation produced severe hepatic injury in the irradiated liver, and the unirradiated liver was stimulated to regenerate, demonstrated by significant increases of serum HGF 30 days after irradiation, increase of mitotic index and the number of PCNA positive hepatocytes 60 days after irradiation. TGF- β 1 was strongly and uniformly expressed in the irradiated liver 90-day-post-irradiation, and it was also expressed slightly in the unirradiated liver region. In summary, partial liver irradiation could stimulate the unirradiated liver to regenerate, and the role of TGF- β 1 in hepatic injury and proliferation needs further investigation.

2. Hepatic regeneration after partial liver irradiation in low dose irradiated liver in rats: The purpose of the study was to investigate whether hepatic regeneration could be stimulated in low-dose irradiated liver by liver injury, induced by high-dose

irradiation. Rats were irradiated with 25 Gy the to right half of the liver, and 0 Gy, 2.5 Gy, 5 Gy, 7.5 Gy to the left half of the liver, respectively. Observation of endpoints in serum or the left half of the liver, including hepatic growth factor (HGF), proliferation cell nuclei antigen (PCNA) and mitosis index (MI) were scheduled on 0-day (before irradiation), 30 days, 60 days, 90 days, and 120 days after IR. Serum HGF and HGF mRNA increased in the protected and low-dose irradiated livers with peaks on d 30. PCNA and PCNA mRNA were raised to their peak on d 60. However, irradiation delayed hepatocyte MI in the irradiated liver, and there was a 30-day delay between the protected and low-dose irradiated livers. In conclusion, liver injury induced by irradiation could stimulate protected livers to regenerate; Low-dose irradiated livers possessed the potential to proliferate with the higher dose received and the stronger regeneration, but irradiation delayed the course of hepatocyte regeneration, and probably over time prevented compensation via hepatocyte regeneration for the loss of hepatocytes.

3. Hepatic Regeneration after Partial Liver Irradiation in Cirrhotic Rats: The objective of the study was to investigate if the right half of the liver was capable of regeneration after sublethal irradiation and whether the irradiation would trigger regeneration of the left half of the liver in the cirrhotic rat model. Cirrhosis of the liver was induced by oral ingestion of thioacetamide (TAA) for 29 weeks. Two experiments were designed into this study. For experiment one, the cirrhotic rats were divided into a control group (without irradiation), and 5 Gy, 10 Gy and 15 Gy groups. The doses of 5 Gy, 10 Gy and 15 Gy were given as a single dose to the right half of the liver of each group respectively with observations of the endpoints scheduled on 0-day (before irradiation), 30 days, 60 days, 90 days and 120 days after irradiation. The results showed that dose-dependent liver proliferation could be stimulated by sublethal partial liver irradiation of 5 Gy to 15 Gy, in both the irradiated and unirradiated parts of the livers, most notable with 15 Gy. Consequently, the irradiation of 15 Gy was chosen as a initial stimulus delivered to the right half of the liver in experiment two in which the cirrhotic rats were divided into a sham (0 Gy) group, 2.5 Gy, 5 Gy and 7.5 Gy groups according to the irradiation dose delivered to the left half of the liver with the right half of the receiving 15 Gy. The same endpoints as those in experiment one were observed every 30 days till 150 days after irradiation. The cirrhotic rats without radiation were chosen to serve as controls. The following endpoints were evaluated: (1). Liver injury: Body weight and Serum ALT, AST, ALP and PA. (2). Liver regeneration: liver index; hepatocyte mitotic index (MI); hepatocyte proliferation index (PI); PCNA labeling index (LI); expression of PCNA mRNA; serum HGF, VEGF, TGF- α , IL-6; and (3) Serum and in situ TGF- β 1.

Results: irradiation of 5 Gy, 10 Gy and 15 Gy to the right half of the liver as well as irradiation of 2.5 Gy, 5 Gy and 7.5 Gy to the left half of the liver with the right half liver receiving 15 Gy could induce hepatic regeneration in both the right and left halves of the livers with indicators of liver regeneration including positive staining for PCNA and PCNA mRNA expression, PI and MI, serum HGF, VEGF and IL-6 levels increasing after IR, and decreased liver injury with a decrease of ALT, AST and ALP and increase of PA and

the expression of TGF- β 1 in serum and in situ. The IR-induced hepatic injury and liver regeneration increased accompanying with a higher IR dose and irradiation of the whole liver.

Conclusion: Dose-dependent and volume-dependent liver injury and liver proliferation could be stimulated by sublethal partial liver irradiation of 15 Gy in both irradiated and unirradiated portions of the liver in cirrhotic rats.

Clinical Usefulness of PIVKA-II in the Detection of Hepatocellular Carcinoma

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Background: Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is a useful tool for surveillance of hepatocellular carcinoma (HCC). The aim of this study was to compare the sensitivity and specificity of serum PIVKA-II with alfa-fetoprotein (AFP) for diagnosing HCC in Korean patients.

Methods: From March 2008 to January 2012, AFP and PIVKA-II were measured from 614 patients with HCC (n=498) or chronic liver disease (n=116). The diagnosis was confirmed histologically or radiologically according to the Korean Association for the Study of Liver Guidelines for the diagnosis of HCC.

Results: The mean age of the patients was 60.1 years, and the male to female ratio was 3.55:1. The cutoff values of AFP and PIVKA-II were defined as 20 ng/ml and 40 mAU/ml. The sensitivity and specificity of PIVKA-II for the diagnosis of HCC were 56% and 83.6%, respectively. When PIVKA-II and AFP were tested together, the sensitivity was increased by 68% and the specificity was decreased by 70.7%. AFP and PIVKA-II showed a statistical correlation in patients with HCC ($P < 0.001$, $r = 0.155$). For the ROC curve in HCC patients, the areas under the curve of AFP and PIVKA-II were 0.709 and 0.729, respectively.

Conclusions: The sensitivity of PIVKA-II and AFP was low. The development of a more sensitive and effective surveillance tool might be needed.

Tumor Vascular Invasion Is an Important Factor for the Prognosis after Hepatic Resection in Hepatocellular Carcinoma

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Background: Recurrence after hepatic resection is one of the most important factors impacting the prognosis and survival of patients with hepatocellular carcinoma (HCC). This study aimed at identifying the prognostic factors affecting the overall survival (OS) and disease free survival (DFS) of patients with HCC after hepatic resection.

Methods: This research was designed as retrospective cohort study and a total of 126 patients who underwent hepatic resection for HCC at Gachon University Gil Medical Center between January 2005 and December 2010 were enrolled. Various clinical, laboratory, and pathologic data were evaluated to determine the prognostic factors affecting the OS and DFS.

Results: The 5-year DFS rate was 26.6%, and the 5-year OS rate was 65%. In the multivariate analysis, preoperative alfa-fetoprotein (> 400 ng/ml), size of tumor (≥ 5 cm), multiple tumors (> 2 nodules), presence of portal vein invasion, modified UICC stage III/IV, and a BCLC stage B/C were the independent prognostic factors affecting the shorter OS. In the multivariate analysis, presence of microvascular invasion, and a BCLC stage B/C were the independent prognostic factors affecting the shorter DFS. Early recurrence within 2 years after operation in 43 patients (34.1%) and presence of microvascular invasion were the independent risk factors for ER in the multivariate analysis.

Conclusions: The presence of vascular invasion was the independent poor prognostic factor affecting the OS, DFS and ER of patients with HCC after hepatic resection. For patients in whom the presence of a vascular invasion after hepatic resection has been identified, close postoperative surveillance for early detection of recurrence and additional treatments are urgently needed.

Re-appraisal of Systemic Doxorubicin Therapy as First-line or Second-line Therapy after Sorafenib in Patients with Advanced Hepatocellular Carcinoma

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Background: In patients with advanced hepatocellular carcinoma (HCC), little is known about systemic cytotoxic-chemotherapy beyond sorafenib. Doxorubicin has long been investigated for HCC treatment and we aimed to assess the efficacy and safety of systemic doxorubicin chemotherapy in patients with HCC.

Methods: We reviewed the medical records of 19 patients who received systemic doxorubicin chemotherapy among patients diagnosed with HCC at the National Cancer Center between October 2000 and December 2011. Doxorubicin was administered intravenously on day 1 at a dose of 60 mg/m² every 3 or 4 weeks.

Results: The median age of the patients given doxorubicin was 51 years and 89.5% were HBV-positive. Fourteen (73.7%) had liver cirrhosis and 89.5% were Child-Pugh class A. According to the modified UICC criteria, 16 of 19 (84.2%) had stage IVb. Systemic doxorubicin was administered for a median of 12.7 months after diagnosis. Nine patients had been treated with sorafenib with a median duration of 5.3 months before systemic doxorubicin therapy. Out of 9 patients given systemic doxorubicin following molecular-targeted therapy, 7 and 8 had received other cytotoxic chemotherapy and transarterial chemoembolization, respectively. The median overall survival was 7.0 months (95% confidential interval [CI]: 4.8–9.3 months); 5.7 months (95% CI: 5.1–6.3 months) in patients who received prior molecular-targeted therapy and 7.2 months (95% CI: 3.5–11.0 months) in patients who did not (P value 0.443). Median cycles of systemic doxorubicin therapy were 2 cycles (range: 1–13 cycles). The reason for discontinuation was progression of disease (44.4%) and intolerable adverse events (22.2%) in patients who had prior molecular targeted therapy. Most common grade 3 and 4 toxicities were neutropenia (44.4%), followed by thrombocytopenia (22.2%).

Conclusions: Systemic doxorubicin chemotherapy may have modest activity with acceptable toxicity in patients with advanced HCC. This study may suggest a consideration of systemic doxorubicin chemotherapy as second-line therapy after sorafenib.

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Optimal Number of Target Lesions for EASL and Modified RECIST Guidelines from a Perspective of Predicting Survival Outcomes in Treatment-naïve Patients with Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization

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Backgrounds/Aims: To date, most studies regarding the optimal number of target lesions for enhancement criteria for hepatocellular carcinoma (HCC) have focused on cross-sectional analyses of concordance. We aimed to determine the optimal number of target lesions for European Association for Study of the Liver (EASL) and modified response evaluation criteria in solid tumors (mRECIST) guidelines in predicting overall survival (OS).

Methods: We analyzed 254 consecutive treatment-naïve HCC patients having at least two measurable target lesions undergoing transarterial chemoembolization (TACE). Kappa-values for intermethod agreement of treatment responses were calculated for comparisons between use of a maximum of one, two, three, or four targets versus use of all target lesions. Prognostic values of radiological assessments according to the number of target lesions for predicting OS were expressed as the C-index.

Results: Based on the EASL and mRECIST guidelines, kappa-values between responses assessing the longest two, three, or four targets and assessing all target lesions were 0.924, 0.977, or 1.000 and 0.907, 0.959, or 1.000, respectively, whereas those between responses assessing only one target and assessing all target lesions were 0.723 and 0.666, respectively. The C-index when measuring the longest one, two, three, four, and all target lesions was similar, ranging from 0.739 to 0.749 for EASL criteria and from 0.750 to 0.759 for mRECIST. From Cox regression analyses, radiological response from each calculation method demonstrated independently significant effects on OS for both guidelines, regardless of the number of target lesions.

Conclusions: Prognostic values for predicting OS were similar regardless of the number of target lesions. Evaluating at least the largest two target lesions rather than only one index lesion should be recommended considering high concordances from cross-sectional analyses.

FibroScan Based Risk Estimation of HBV-related HCC Occurrence: Development and Validation of a Predictive Model

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Background/ aims: Liver stiffness measurement (LSM) using transient elastography can predict hepatocellular carcinoma (HCC) development in patients with chronic hepatitis B (CHB). The aim of this study was to develop and validate a novel LSM-based predictive model for HCC development.

Methods: A total of 1,250 patients with CHB with baseline LSM were recruited between May 2005 and December 2007. If a given variable was previously known to contribute to HCC development (HBV DNA $\geq 20,000$ IU/L) or it was selected as an independent predictor of HCC development from multivariate analysis in our study (age, male gender, and LSM value), it was incorporated into the new predictive model as a constituent variable. The predictive model of HCC development was constructed based on the Cox proportional hazards model. We estimated baseline disease-free probabilities at 3 years. Model validation had two steps: discrimination and calibration.

Results: The mean age of our cohort was 50 years (760 men). The median LSM value was 7.7 kPa and high HBV DNA $\geq 20,000$ IU/L was identified in 349 (31.4%) patients. During the median follow-up of 30.7 years, 56 patients developed HCC. On multivariate analysis, age, male gender, and LSM values were selected as independent predictors of HCC development (all $P < 0.05$) whereas HBV DNA $\geq 20,000$ IU/L showed only borderline statistical significance ($P = 0.0659$). We developed a predictive model for HCC development using these four variables which showed a fairly good discrimination capability, with an area under the receiving operator characteristic (AUROC) of 0.806 (95% confidence interval, 0.738–0.874).

Probability = $1 - P^A$

$A = \exp(0.05306 \times \text{age} + 1.106 \times \text{male gender} + 0.04858 \times \text{LSM values} + 0.50969 \times \text{HBV DNA} \geq 20,000 \text{ IU/L})$

For the assessment of discrimination, we used 10-fold cross-validation. The AUROCs remained largely unchanged between iterations, with the average AUROC of 0.813 (95% confidence interval, 0.804–0.822). When we plotted a calibration chart to predict and observe the risk of HCC development, it predicted a risk of HCC development which calibrated fairly well with the observed risk with a correlation coefficient of 0.905 ($P < 0.001$).

Conclusion: A simple predictive model for HCC development was developed and validated in our study. This novel model accurately estimates the risk of HCC development in patients with CHB with an AUROC of 0.806.

Antiviral Efficacy and Safety of Telbivudine in Hepatitis B Virus-related Hepatocellular Carcinoma

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Background: Antiviral therapy with nucleos(t)ide analogues has been shown to improve liver function in patients with chronic hepatitis B (CHB). However, the antiviral efficacy and safety of telbivudine therapy in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) patients has not been evaluated.

Methods: Of 223 CHB patients consecutively treated with 600 mg/day telbivudine for at least 6 months, 80 had HCC (HCC group; A) whilst 143 did not (non-HCC group; B). During telbivudine treatment, biochemical, virologic and serological parameters were monitored regularly.

Results: There was no significant difference in baseline characteristics between the two groups. The median duration of telbivudine treatment was 16.1 months. At Month 21, a median change in serum HBV DNA from baseline was similar between the groups (A vs B: -3.5 vs -4.0 log₁₀ IU/ml, $P = 0.671$). The cumulative complete virologic response (serum HBV DNA < 12 IU/ml) was also comparable (A vs B: 57.5% vs 65.7%, $P = 0.318$). ALT normalization was 63% in the HCC group and 81% in the non-HCC group. HBeAg seroconversion was 5.7% in the HCC group and 4.4% in the non-HCC group. The cumulative rate of viral breakthrough was higher in the non-HCC group, but this difference was not significant (17.5% vs 29.3%, $P = 0.055$). There was no hepatitis flare in either group, suggesting that telbivudine effectively prevented HBV reactivation during anti-HCC treatment. Interestingly, telbivudine treatment improved liver function, as indicated by an increase in serum albumin level ($P < 0.001$) and decrease in Child-Pugh scores ($P < 0.001$). No serious side effects were seen in any patient.

Conclusion: Telbivudine had comparable antiviral efficacy and safety in both HCC and non-HCC patients. During telbivudine treatment, there was no HBV-reactivation-induced hepatitis flare due to anti-HCC treatment; moreover, liver function improved.

Effect of PTEN Gene Polymorphisms on the Development of Hepatitis B Virus Associated Hepatocellular Carcinoma

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Background/Aim: Phosphatase and tensin homologue (PTEN) is known as a tumor suppressor for many tumor types and the expression of PTEN is reduced or absent in almost half of all hepatocellular carcinomas (HCCs). In the present study, we investigated the association between single nucleotide polymorphisms (SNPs) in the PTEN gene and the development of HCC in hepatitis B virus infected patients.

Methods: Between March 2002 and December 2006, 139 HCC patients and 99 chronic HBV carriers without HCC were enrolled in this study. We analyzed SNPs at six polymorphic sites in the PTEN gene at positions –7748 G>T, –4017 A>G, +21246 C>T, +2–97 C>T, +80492 A>G and +96024 C>T in the study subjects.

Result: The PTEN –4017 A allele (OR; 0.590, CI; 0.409–0.849, $P=0.004$), the +2–97 C allele (OR; 0.492, CI; 0.320–0.755, $P=0.001$), the +80492 A allele (OR; 0.593, CI; 0.415–0.847, $P=0.001$) and the +96024 C allele were significantly associated with the development of HCC. Additionally, the haplotype 1 (GGCTGT) showed preventive effects on HCC occurrence.

Conclusions: This study indicates that PTEN gene polymorphisms are associated with HBV related HCC development.

The Clinical Significance of CD163 is Higher Than CD68 in Patients with Hepatocellular Carcinoma Who Underwent Curative Resection

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Background: Hepatocellular carcinoma (HCC) is one of the most prevalent cancers in the world. We have found that the abundance of peritumoral CD68+macrophages was associated with poor prognosis for HCC patients after curative resection. However, CD68 staining cannot discriminate the protumoral or tumoricidal subpopulations from pan-macrophages.

Methods: The CD163+and CD68+cells in tumor and peritumoral liver tissue were detected with immunocytochemistry in a cohort of 295 HCC patients and their correlation with the clinicopathological features were analyzed. The preoperative plasma concentration of the soluble form of CD163 (sCD163) was also

detected in another cohort of 107 HCC patients with an ELISA.

Results: We found that the count of CD163+ cells correlated well with, and much higher than that of, CD68+ cells in both tumor and peritumoral liver tissues, respectively, which was consistent with the observation from immunostaining with CD68 or CD163 antibodies on consecutive sections. A higher count of peritumoral CD68+ cells was associated with poor recurrence-free survival and overall survival ($P = 0.004$ and $P = 0.001$, respectively), whereas the counts of tumor and peritumoral CD163+ cells did not. In another cohort of 107 HCC patients, plasma concentration of the soluble form of CD163 (sCD163) was associated with active hepatitis-related factors (e.g., serum alanine aminotransferase, serum aspartate aminotransferase, γ -glutamyl transpeptidase, and serum alkaline phosphatase).

Conclusions: The CD163+ cell count is of limited significance for predicting the prognosis of patients with HCC. Plasma sCD163 was more likely a factor related to active hepatitis rather than tumor-related features.

Combined Radiofrequency Ablation and Ethanol Injection with a Multi-pronged Needle for the Treatment of Medium and Large Hepatocellular Carcinomas

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Background: To evaluate the efficacy of combined radiofrequency ablation and ethanol injection with a multi-pronged needle in the treatment of medium (3.1–5.0 cm) and large hepatocellular carcinomas (HCCs).

Methods: 50 patients (mean age, 55.3 years; age range, 30–81 years) with a total of 52 (3.1–7.0 cm) HCC nodules were enrolled in this prospective study. The study was approved by the ethics committee of the hospital and informed consent was signed by each patient. The average size of the tumors was 3.8 ± 0.8 cm by 3.1 ± 0.7 cm. The treatment was performed percutaneously under ultrasound guidance. Initial tumor response was assessed on 1-month CT scans with the modified Response Evaluation Criteria in Solid Tumors (mRECIST) for HCC. The follow-up protocol included imaging examinations at 3-month intervals.

Results: The average volume of injected ethanol was 14.4 ± 4.3 ml (range, 9–30 ml). The average number of RFA electrode insertions was 1.8 ± 0.8 (range, 1–4). The mean size of the ablation zone was 5.1 ± 0.9 cm (range, 2.9–6.7 cm) $\times$ 4.2 ± 0.8 cm (range, 3.6–8.6 cm). The rate of initial local complete response (CR) ac-

cording to mRECIST was 94.2% (49/52). After additional treatment, technical success was achieved in all (100%) nodules. After a mean follow-up period of 15.6 ± 5.3 months (range, 6.5–25.0 months), local tumor progression was observed in 7 (13.5%) of the 52 tumor nodules with CR. Therefore, the rate of sustained local CR was 86.5% (45/52). Distant recurrence developed in 38% ($n = 19$) of patients. There were no treatment-related deaths, and major complications were observed in 3 (6%) patients (1 liver abscess, 1 massive hemoperitoneum, and 1 massive ascites due to liver function damage). Minor complication included asymptomatic intraabdominal hemorrhage ($n = 4$), pleural effusion ($n = 1$), and bilomas secondary to bile duct damage ($n = 2$). The 1-year and 2-year overall survival rates were 90.8% and 88.1%, respectively.

Conclusion: The combination of RFA and ethanol injection with a multipronged needle in the treatment of medium and large HCCs is safe and effective. It can provide a high rate of local tumor control for the treatment of patients with medium and large size HCCs.

A Precise Safety Margin of 1.0 cm is Required for Best Local Efficacy of Radiofrequency Ablation of Hepatocellular Carcinoma: Assessment with a Novel Three Dimensional Reconstruction Software

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Background: To analyze the relationship between the safety margin and local tumor control of radiofrequency (RF) ablation in treatment of small hepatocellular carcinoma by reconstructing and fusing 3-D images of tumor zones and ablation zones with a three dimensional reconstruction software.

Methods: From March 2011 to December 2011, 69 patients (10 female and 59 male) with 95 hepatocellular carcinoma nodules less than 5 cm admitted to the Division of Interventional Ultrasound, The First Affiliated Hospital of Sun Yat-sen University were enrolled in the study. The study was approved by the Ethics Committee of the hospital and informed consent was signed by all patients. All nodules were treated using RF ablation with curative intention. A 64-row enhanced CT scan was performed before and 1 month after treatment. CT data were processed with three dimensional reconstruction software. Three dimensional tumor and ablation zones were reconstructed into 3-D images and fused to show the safety margins for accurate measurement. Ablation margin, tumor size, tumor volume and heat sink effect due to adjacent hepatic vessels were assessed with a multivariate analysis.

Results: Follow up was conducted on all patients for 6.3 months (2.0–10.9 months). The reconstructed ablation zone cov-

ered the original tumor zone completely in each tumor nodule, but the safety margins varied in all directions. The complete ablation rate was 97% (92/95). LTP was found in 4.2% (4/95) nodules. A safety margin of 5.5 ± 2.0 mm (0.9–8.0 mm) was found in those specimens where incomplete ablation or LTP had occurred. A safety margin < 1 cm is the sole significant factor related with insufficient local efficacy.

Conclusion: The use of three dimensional reconstruction software makes it possible to calculate the precise safety margin of RF ablation. To achieve the best local efficacy, a safety margin of at least 1.0 cm is necessary.

Feasibility of α -fetoprotein as A Screening and Diagnostic Tool for Hepatocellular Carcinoma (Multicenter Study)

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Background: Recent systematic reviews show that the quality of evidence supporting the use of AFP as a diagnostic and screening test for hepatocellular carcinoma (HCC) is limited. The Korean Liver Cancer Study Group recommended the cutoff value of 200 ng/ml as a diagnostic tool. The aim of this study was to evaluate the feasibility of AFP as a screening and to explore the optimal level of AFP for HCC diagnosis

Methods: We retrospectively reviewed the medical records of HCC (all cases were biopsy proven) and cirrhosis patients whose data were available in 3 hospitals. A total of 1,560 patients were classified into three groups: 564 were cirrhosis controls, 766 were patients with early stage HCC ($n = 123$ very early, $n = 643$ early) and 230 were patients with late stage HCC. The performance of AFP in the three groups was compared with each other. The AFP sensitivity, specificity, positive and negative predictive values were calculated.

Results: The mean age was 54 years in patients, and 56 years in controls. Males were dominant in both groups (patient, 81.1% vs. control, 65.7%). The mean AFP was significantly higher in patients with overall HCC (early, late) than in those with liver cirrhosis. [early HCC, 2,769 ng/ml (1–200,000), late HCC, 9,818 ng/ml (1–639,100), control, 20 ng/ml (0.61–1,753)]. ($p < 0.0001$) The area under the receiver operating characteristic curve for those overall HCC patients with cirrhosis was 0.78. The sensitivity, specificity, and PPV of AFP were 52.6%, 87.7%, and 88.3% at a cutoff of 20 ng/ml; 36.9%, 95.9% and 94.1% at a cutoff of 100 ng/ml and 29.5%, 98.0% and 96.3% at a cutoff of 200 ng/ml, respectively. As a screening tool for HCC, a cutoff of 100 ng/ml is more

sensitive than 200 ng/ml with equivalent specificity and PPV. The optimal AFP cutoff value was 8.2 ng/ml leading to a sensitivity of 66.9% and a specificity of 75.7%.

Conclusions: The results of our study cautiously suggested that the optimal cut-off value of AFP in the diagnosis of HCC might be switched from 200 ng/ml to 100 ng/ml.

Effectiveness of using Gd-EOB-DTPA-enhanced MRI for confirmation of hepatocellular carcinoma in Thailand and Korea as compared to extracellular contrast media-enhanced MRI and 3-phase MD-CT

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Object: To analyze the economic outcomes of Primovist MRI (Gd-EOB-DTPA-enhanced MRI) compared to extracellular contrast media-enhanced MRI (ECCM-MRI) and three-phase multi-detector CT (three-phase-MDCT) as initial imaging procedures in patients with suspected hepatocellular carcinoma (HCC) in South Korea and Thailand.

Materials and Methods: This economic evaluation was based on a decision-tree model which displayed the clinical pathway of patients with suspected HCC from the initial imaging procedure until the first treatment decision. The payer relevant costs of Gd-EOB-DTPA-enhanced MRI, ECCM-MRI and three-phase-MDCT as the first line imaging for suspected HCC and their subsequent costs until the first treatment decision were compared. The considered input data (probabilities and resource consumptions) were estimated and validated by clinical experts. Costs for diagnostic and treatment options were derived from published sources.

Results: In Korea, the total costs per patient until first treatment using Gd-EOB-DTPA-enhanced MRI as the first imaging in patients with suspected HCC, amounted to 3,098 USD. This is slightly higher than the costs for three-phase-MDCT per patient (+4 USD), but lower than the costs of ECCM-MRI (–306 USD).

In Thailand, Gd-EOB-DTPA-enhanced MRI was the least costly alternative compared to ECCM-MRI and three-phase-MDCT (702 USD per patient). Cost savings per patient amounted to –228 USD compared to three-phase-MDCT and –170 USD compared to ECCM-MRI.

Conclusion: The use of Gd-EOB-DTPA-enhanced MRI compared to ECCM-MRI and three-phase-MDCT as the initial imaging in patients with suspected HCC led to relevant cost savings for the statutory health insurance in both countries.

Clinical Characteristics and Outcome of Hepatocellular Carcinoma in Korea: Results from a Random Sample Audit of the Statutory Nationwide Cancer Registry

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Background: Given the high incidence and mortality rate of hepatocellular carcinoma (HCC), ensuring the high quality of cancer registry data is important for the improvement of the health service. Registries based on voluntary reporting often lack case completeness and may cause selection bias. A statutory Korean nation-wide cancer registry has case completeness and provides accurate information on HCC incidence, but lacks data completeness and provides limited information about HCC characteristics.

Methods: A total of 4,522 HCC patients corresponding to about 15% of all 31,521 HCC registrants to the Korean nation-wide cancer registry between 2003 and 2005 were randomly selected. Data for their clinical characteristics and outcomes were collected through reabstracting audits from 32 nation-wide hospitals.

Results: Mean The mean patient age of patients was 57±11, and 81% were male. Hepatitis B virus, hepatitis C virus, and alcohol were presumed causes of HCC in 72%, 12%, and 10% of patients, respectively. About 64% of patients had Child-Pugh class A liver function. TNM stages of patients were I in 11%, II in 43%, III in 28%, and IV in 18%. Initial treatment modalities were transarterial embolotherapy in 49.5%, surgical resection in 9.5%, local ablation in 6.9%, and liver transplantation in 0.7%. Median The median survival time was 17 months. The 1-, 3-, and 5-year survival rates of these patients (55%, 35%, and 26%, respectively) were significantly worse than those of patients in the voluntary registry of the Korean Liver Cancer Study Group (67%, 46%, and 34% respectively).

Conclusions: About half of HCC patients are diagnosed at advanced stages in Korea. Curative intent treatments are rarely applied to patients. The data from a random sample audit of a nation-wide HCC registry provides unbiased and accurate information of clinical characteristics and outcome of HCC in Korea.

Treatment Strategy for Hepatocellular Carcinoma with Major Portal Vein or Inferior Vena Cava Invasion

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Background: The prognosis of patients with hepatocellular carcinoma (HCC) invading the main trunk of the portal vein and the inferior vena cava is poor. The best strategy for treatment is not well known.

Methods: We retrospectively reviewed the medical records of 756 patients treated for HCC between 2006 and 2011. One hundred and fifty-six (20.6%) of these patients had HCC, with a tumor thrombus invading the main trunk or the first-order branch of the portal vein, or the inferior vena cava. Fifty-two patients underwent hepatectomy and 104 patients underwent transcatheter arterial chemoembolization (TACE) and/or radiotherapy or/and Sorafenib. We specifically focused on these 52 patients to describe our results of surgical treatment for advanced HCC.

Results: Among the 52 patients who underwent hepatectomy, preoperative TACE was performed in 32 patients. Twenty patients were identified as having a tumor size reduction or necrosis of 50% or higher (TE3) following TACE. The median operative duration was 303 minutes. Postoperative morbidity and mortality rates were 11.5% and 1.9%, respectively. The 5-year survival rate after hepatectomy was 37.4%, which was better than that of patients after TACE alone. The response after preoperative TACE (hazard ratio 4.88; 95% CI, 1.15 to 20.77, $p=0.025$), resection margin (hazard ratio 6.45; 95% CI, 1.27 to 32.26, $p=0.032$), and tumor diameter (hazard ratio 4.25; 95% CI, 1.01 to 11.98, $p=0.049$) were associated with significant favorable preoperative prognostic factors for survival using the multivariable Cox Regression. Patients with tumors smaller than 10 cm and TE3 effect had a more favorable survival than patients with tumors 10 cm or larger and who did not have a good TACE outcome.

Conclusions: A combination of aggressive surgical treatment and effective preoperative TACE treatment for HCC with major vascular invasion may be beneficial for selected patients with advanced HCC.

Enhancement Features of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma on Contrast-enhanced Ultrasound—based on 1239 Patients from 10 Centers in China

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Background: Contrast-enhanced CT and MRI play important roles in the non-invasive diagnosis of hepatocellular carcinoma (HCC). Contrast-enhanced ultrasound (CEUS) also showed a very high performance in characterization of focal liver lesions, but with limited ability to differentiate HCC from intrahepatic cholangiocarcinoma (ICC).

Objective: To summarize the enhancement patterns of HCC and ICC on CEUS and find features useful for differential diagnosis between these two entities.

Methods: A total of 1239 patients (HCC=1120, ICC=119) with pathological confirmation were examined with real-time CEUS using a sulfur hexafluoride-filled microbubble contrast agent. The enhancement appearances of lesions were analyzed.

Results: The typical pattern of malignant tumors, defined as arterial hypervascularity followed by washout, was observed in 88.7% (993/1120) of the HCC and 83.2% (99/119) of the ICC lesions, without significant difference between these two entities ($p=0.079$). Among the lesions showing the typical pattern, washout during the portal phase was more common in ICC (99.0%, 98/99) compared with HCC (78.2%, 777/993) ($p<0.001$). As to the enhancement appearance, peripheral rim-like enhancement was observed in 62.6% (62/99) of ICCs which was significantly higher than in the HCCs (0.7%, 7/993) ($p<0.001$). For ICC, pe-

peripheral rim-like enhancement was even more common in lesions >5 cm in diameter (68.5%, 50/73) than those <5 cm (46.2%, 12/26) ($p=0.043$).

Conclusions: Both HCC and ICC may show the typical enhancement pattern of malignant tumor on CEUS. Portal phase washout and peripheral rim-like enhancement are much more common in ICC than HCC, which may provide a useful clue for differentiation.

Fgl2/prothrombinase Contributes to HCC Tumor Growth and Angiogenesis through The MAPK Pathway

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Fibrinogen-like protein 2 (fgl2)/ prothrombinase has been identified as a procoagulant protein which directly generates thrombin from prothrombin without activation of the conventional coagulation cascade. Fgl2 has been found to be overexpressed in a number of human malignant tumors including human hepatocellular carcinoma (HCC), however, its functional role in malignancy remain largely unexplored. To define the role of tumor cell-associated fgl2 in tumor growth and tumor angiogenesis, we established a fgl2 stably knocked down HCCLM6 cell line, a highly aggressive HCC derived cell line in which fgl2 expression was found upregulated in response to tumor necrosis factor- α (TNF- α) stimulation in a dose-dependent manner. Consequently, fgl2 depletion in HCCLM6 cells led to delayed tumor growth and vascularization of HCC xenografts in nude mice. These changes were associated with decreased phosphorylation of extracellular signal-regulated kinases (ERKs) but not p38 mitogen-activated protein kinase (p38-MAPK) *in vivo*. *In vitro*, fgl2 depleted HCCLM6 cells exhibited decreased proliferation and pro-angiogenic factors expression following TNF- α stimulation, associated with a higher susceptibility to TNF- α induced apoptosis. One mechanism underlying this phenomenon that we discovered was that endogenous fgl2 enhanced TNF- α induced MAPK pathway activation in HCCLM6 cells through both a thrombin dependent and independent manner. However, exogenous recombinant fgl2 was found to induce phosphorylation of ERK and p38-MAPK only in a thrombin dependent manner. Collectively, our data suggest that fgl2 expression in tumors is regulated by inflammatory cytokines, and that it contributes to tumor growth and tumor angiogenesis through both thrombin dependent and independent activation of the MAPK pathway.

Percutaneous Ablative Therapies of Recurrent Hepatocellular Carcinoma after Hepatectomy: Proposal of a Prognostic Model

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Hepatectomy offers the best hope of cure for patients with hepatocellular carcinoma (HCC), but is associated with a high incidence of tumor recurrence. Percutaneous ablative therapy (PAT) has been widely applied for recurrent HCCs after liver resection and has represented a valuable modality in prolonging patient survival. However, it still remain unclear what category of patients could benefit best from the treatment. The present study retrospectively analyzed a series of post-hepatectomy recurrent HCCs treated with PAT, and aimed at introducing a prognostic model which may be helpful for selecting the appropriate therapeutic strategy for recurrent HCC. From October 1997 to May 2010, 288 patients with recurrent HCC after liver resection were treated in our hospital with ultrasound guided percutaneous ethanol ablation (EA), radiofrequency ablation (RFA), microwave ablation (MWA) or EA combined with RFA. The 1-, 3-, 5- and 7-year survival rates after ablation were 71.1%, 37.8%, 20.7%, 14.2%, respectively, with a median survival time of 24.0 months. A multivariate analysis using the Cox regression model revealed that the interval between recurrence and initial hepatectomy, number of tumors, the greater diameter of the tumor and the BCLC stage at initial hepatectomy were independent prognostic factors for long-term survival. By quantifying and integrating the afore-mentioned prognostic factors, a prognostic model was created. A scoring system from 1 to 3, in which a lower score was associated with a more favorable stratum of each prognostic factor, was proposed, and the summation of 4 prognostic factors, which we called the prognostic score, ranged from 4 to 10. The prognostic score was classified into three strata, designated as prognostic class A (score 4 and 5), B (score 6 and 7) and C (≥ 8). Use of the prognostic model for analysis of post-ablation survival demonstrated that the patients had 1-, 3-, 5- and 7-year survival rates of 93.6%, 62.8%, 39.4% and 26.9% in class A, 71.0%, 36.9%, 15.5% and 7.2% in class B, and 40.5%, 5.5%, 0% and 0% in class C, respectively ($p=0.0000$). The 1-, 3-, 5-, 7-year and 10-year overall survival after initial hepatectomy were 100%, 82.4%, 66.3%, 52.1% and 36.4% in class A, 82.5%, 51.6%, 34.8%, 20.7% and 6.6% in class B, and 63.4%, 11.9%, 7.8%, 0% and 0% in class C, respectively ($p=0.0000$). The prognostic model presented could clearly predict different outcomes for patients with recurrent HCC after hepatectomy treated with PAT. For the patients in class A, PAT alone may be expected to yield a good long-term survival and is strongly recommended given its minimal-invasiveness and easy repeatability. For the patients in class B, PAT may probably not

be adequate and repeat hepatectomy is the first choice when feasible, or multidisciplinary treatments are needed. For the patients in class C, PAT had difficulty in improving the long term outcome and other treatments, such as trans-arterial chemoembolization (TACE) and systemic therapy (e.g., molecular target therapy) need to be taken into consideration. Admittedly, the main limitation of the current study is that it was retrospective in nature therefore large-scale prospective clinical trials are needed to verify the validity of the prognostic model proposed.

M2 Macrophages Promote Tumor Growth and Invasion in Hepatocellular Carcinoma

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Background and Aim: Alternative activated macrophages (M2) have been demonstrated to promote tumor growth and invasiveness in many cancers except hepatocellular carcinoma (HCC), which is the fifth most frequent malignancy worldwide with high mortality and recurrence rates. Understanding the properties and functions of M2 macrophages is essential for improving both the prognosis and therapeutic strategies of HCC. In this study, we investigated the role of M2 macrophage on HCC tumor progress.

Materials and Methods: M2 macrophages in HCC patients were detected with immunohistostaining using a common macrophage marker [CD68] and a M2 specific marker [CD163]. The expression pattern of M2 macrophages was further correlated with clinical pathological parameters in terms of tumor size, staging, survival and metastasis. The mRNA expression of M2 specific scavenger receptor A (SR-A) and mannose receptor (MR) measured with quantitative real-time RT-PCR were also compared. The function and characteristics of M1 (anti-tumor) and M2 (pro-tumor) polarized macrophages derived from the human THP-1 cell line were examined in a series of in vitro functional studies. The roles of M1 and M2 macrophages in liver tumor growth and metastasis were further validated in an orthotopic liver cancer nude mouse model.

Results: In the clinical association study, both the numbers of CD68-positive and CD163-positive cells in non-tumor but not the tumor region significantly correlated with poor overall survival (CD68: $p=0.023$; CD163: $p=0.042$), large tumor size (CD68: $p=0.028$; CD163: $p=0.027$) and high recurrence rate (CD68: $p=0.049$; CD163: $p=0.034$). Consistently, mRNA expression of SR-A and MR mRNA also significantly correlated with overall survival, tumor size and recurrence rate ($p<0.05$). In the in vitro functional studies, by co-culturing with M2 and M1 polarized macrophages, the HCC cell line – MHCC97I showed a 1.5-fold increase and a 3-fold decrease, respectively, in the growth rate compared with the control ($p=0.019$). In addition, co-culture with M2 but not M1 polarized macrophages

induced the release and activities of matrix metalloproteinase-9 (MMP-9) and MMP-2, which subsequent promoted invasiveness of the HCC cell line – MHCC97I detected by cell migration and invasion assays. In the orthotopic liver tumor nude mouse model, portal vein injection of M2 macrophages induced a 30% increase in the tumor size compared with the control group. On the contrary, M1 macrophage injection decreased the tumor size by 90%. Furthermore, a higher lung metastatic rate (57%) was also noted in M2 treated group compared with the control group (25%, $p<0.05$).

Conclusions: Clinical analysis and both the in vitro and in vivo studies presented here collectively illustrated that M2 macrophages promoted tumor growth and invasion in HCC. On the other hand, tumor suppression phenotypes of M1 macrophages were also shown. Inhibition or M1 phenotypic conversion of M2 macrophages in situ represents a novel approach for treating this disease.

Utility of Gadoteric Acid-Enhanced MRI in the Surveillance for Postoperative Recurrence of Hepatocellular Carcinoma

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Background/Aim: Gadoteric acid-enhanced magnetic resonance imaging (MRI) is shown to be superior to computed tomography (CT) in detection and characterization of liver lesions. The objective of the present study was to investigate the utility of MRI in surveillance for postoperative recurrence of hepatocellular carcinoma (HCC).

Methods: This retrospective study analyzed a total of 147 patients who underwent surveillance with a combination of CT and annual gadoteric acid-enhanced MRI after hepatectomy. The subjects in whom MRI was performed because of suspected recurrence based on increasing alpha-fetoprotein levels were excluded. In this cohort, the detection rate of each HCC recurrence on MRI and CT was evaluated and recurrent HCC characteristics were compared according to the detection test.

Results: Sixty-two patients had recurrent HCC. Among them, detection in 9 was with MRI and 29 with CT. In the baseline characteristics of the patients with recurrent HCC, there were no significant differences according to the detection test regarding age, gender, etiology of liver disease, platelet count, liver function tests, Child-Pugh class, alpha-fetoprotein level, fibrosis stage, Edmondson-Steiner class, modified UICC stage, or hepatectomy extent. The HCC recurrence detection rates based on MRI and CT were 4.8% (9/180) and 4.3% (29/580), respectively, on the per test basis ($P=0.764$). However, in the population with a follow-up of ≥ 12 months, the detection rates based on MRI and CT were 4.3% (7/150) and 1.5% (19/400), respectively ($P=0.035$). The

likelihood of HCC recurrence detection in the following surveillance after a negative result based on MRI or CT was 1.5% (2/133) and 6.0% (36/596), respectively ($P=0.033$). The recurrent HCCs detected with MRI were smaller than that detected with CT (size <2 cm, 100% vs. 65.5%, $P=0.040$).

Conclusions: Our data suggest that gadolinium-enhanced MRI has a higher detection rate for postoperative HCC recurrence than CT. Surveillance with combination of CT and MRI may identify recurrent HCC at an earlier stage than with CT alone.

Early Recurrent Cases of Small-sized Hepatocellular Carcinoma after Hepatectomy

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Introduction: The prognosis for hepatocellular carcinomas (HCCs) that are smaller than 3 cm is good: the 5-year survival rate has been reported as 80%. However, some patients have recurrences within a year. In this series, we investigated the clinical features of patients with early recurrence of small-sized HCCs after hepatectomy.

Patients and Methods: From Jan. 2005 to Dec. 2008, 177 patients with HCC underwent surgery in our hospital, and 49 of them had tumors smaller than 3 cm. Sixteen (32%) of 49 patients had recurrences within a year. The clinico-pathological features of the 16 patients were investigated.

Results: Three patients had de novo cancers in the remnant liver. Seven of the other 13 patients were so advanced that they had intrahepatic metastasis and severe invasion of the portal vein or bile duct. In another 6 patients, in whom the disease was not so advanced, the tumors were completely resected but recurrences occurred within a year. Four of them were female, 4 had several tumors which were all de novo HCCs and 3 had other treatments, such as ablation and chemotherapy, before operation. Ten of the 13 patients, which included the 7 advanced patients, had tumors belonging to the 'simple nodular type with extranodular growth' or the 'confluent multi-nodular type', according to the classification of macroscopic types of HCC of the Liver Cancer Study Group of Japan.

Discussion and Conclusions: HCCs with macroscopic findings of simple nodular type with extranodular growth or confluent multi-nodular type are often accompanied by vascular invasion or intrahepatic metastasis, even if they are smaller than 3 cm. Adjuvant therapy or liver transplantation should be considered for such patients after operation. Based on our patients, some patient factors like females and patients with several tumors tended to have recurrence within a year.

Changes in Arterio-portal Shunts after Transcatheter Arterial Chemoembolization and Radiation Therapy for Hepatocellular Carcinoma with Portal Vein Tumor Thrombosis

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Background: Arterioportal (AP) shunts are frequently associated with advanced hepatocellular carcinoma (HCC) and there is a lack of data regarding the changes in arterioportal (AP) shunts after radiation therapy (RT) for HCC. We investigated the prevalence of AP shunts in treatment-naïve HCC patients with portal vein tumor thrombosis (PVTT), and evaluated the changes in AP shunts after transcatheter arterial chemoembolization (TACE) followed by RT.

Methods and Materials: A total of 54 patients who had treatment-naïve HCC and PVTT were treated with TACE followed by RT between June 2008 and December 2010. RT was delivered as 30 to 45 Gy (median 35 Gy) in 2 to 4.5 Gy (median 3.5 Gy) per fraction using a 6, 10, or 15 MV X-ray. Tumor response was assessed with dynamic CT at 1 month after completing RT using the modified Response Evaluation Criteria in Solid Tumor (RECIST) guidelines. Angiographic images of TACE before and after RT were reviewed to investigate the AP shunt.

Results: At the 1st angiography, 33 of 54 patients (61%) had an AP shunt. Thirty-two of 33 patients had a 2nd angiography and were evaluated for a change of the AP shunt after RT. The AP shunt improved in 20 patients (63%) after RT. At 1 month after completing RT, complete response was obtained in 5 patients (9%), partial response in 21 (39%), stable disease in 25 (46%), and progressive disease in 3 (6%). Improvement in the AP shunt was achieved in 86% of patients with a response and 44% of patients without a response, representing a significant difference ($p=0.017$).

Conclusion: In conclusion, the AP shunt is frequently associated with HCC patients with PVTT. AP shunts could be effectively obliterated with TACE and RT.

A Two-week Schedule of Hypofractionated Radiotherapy in Patients with Small Hepatocellular Carcinoma: Efficacy and Clinical Outcomes.

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Background: For small hepatocellular carcinoma (HCC) when established curative treatment cannot be applied, stereotactic body radiotherapy (SBRT) has been used as a non-invasive alternative treatment modality. However, this SBRT may not be safe if normal liver volume is limited or the tumor is located around critical normal organs. We therefore applied hypofractionated radiotherapy under these situations, and evaluated the clinical outcomes of this modality.

Methods: Between December 2008 and August 2011, 26 patients (28 lesions) with HCC were treated with hypofractionated radiotherapy. Inclusion criteria were HCC not suitable for surgery or other local ablative therapy, tumor size <6 cm, adequate hepatic function, HCC located 1–2 cm from a critical organ, and no evidence of vascular invasion. A dose of 4–5 Gy per fraction was given to a total dose of 40–50 Gy for 2 weeks. Local failure was defined as a recurrence in the treated lesion, and regional failure was a recurrence within the liver outside the treated lesion. Radiation-induced hepatic toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.0.

Results: The median follow-up was 17 months. The response rate was 78%, with 11 complete responses (39%) and 11 partial responses (39%) at 3 months after the end of hypofractionated RT. The overall survival rates at 1- and 2 year were 86.2% and 58.2%, respectively. Local failure was observed in two (7%) of 28 lesions. Regional failure and distant metastasis were observed in 14 (54%) and 6 (23%) patients, respectively. Grade ≥3 hepatic toxicity was observed in 1 (3.8) patient.

Conclusion: Hypofractionated RT for small HCC was feasible and showed good local control. This dose-schedule can be used as an alternative treatment scheme for small HCCs with limited normal liver volume or HCCs located close to critical normal organs.

Observations of Hepatocellular Carcinoma (HCC) Management Patterns from the Global HCC BRIDGE Study: Characterization of the Full Study Population

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Background: Hepatocellular carcinoma (HCC) is a major worldwide health problem. The global HCC BRIDGE study is the first large-scale, observational study to document the real-world experience of HCC patients from diagnosis to death.

Methods: This longitudinal cohort study (started March 2009) includes HCC patients newly diagnosed between January 2005 and June 2011 and treated at major medical centers, with data collected retrospectively and prospectively as recorded in patient charts.

Results: At the second interim analysis (March 2012), 17,230 treated HCC patients were enrolled at 38 sites in China (n=8683; 50%), three other Asian countries (Taiwan, South Korea, and Japan; n=3329; 19%), Europe (n=2956; 17%), and North America (n=2262; 13%). The mean age ranged from 52 (China) to 65 years (Europe); approximately 80% were male. The most common HCC risk factor was HBV in China (77%) and other Asian countries (61%), and HCV in Europe (47%) and North America (39%). The most common BCLC stage at diagnosis was stage C in China (55%), Europe (50%), and North America (42%), and stage A in other Asian countries (43%). First recorded treatments in China, other Asian countries, Europe, and North America included resection (32%, 33%, 16%, 19%), transplantation (1%, 2%, 3%, 2%), TACE (51%, 35%, 27%, 34%), PEI/RFA (4%, 16%, 30%, 19%), other locoregional therapy (6%, 3%, 9%, 7%), sorafenib (1%, 4%, 7%, 8%), and other systemic therapy (1%, 4%, 1%, 1%), respectively. Median survival from the first HCC treatment in China was 19 months, not reached in other Asian countries, 21 months in Europe, and 35 months in North America.

Conclusions: As the largest study of its type, the global HCC BRIDGE study provides insights into HCC disease characteristics and patient management. Differences in risk factors among regions confirm well-known trends, while other observed differences (e.g., treatment variations) may be related to country/site-specific practices and patient characteristics.

Brivanib Versus a Placebo in Patients with Advanced Hepatocellular Carcinoma (HCC) who Failed Or Were Intolerant to Sorafenib Treatment: Results from the Phase 3 Brisk-ps Study

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Background: Brivanib is a selective tyrosine kinase inhibitor of the vascular endothelial growth factor and fibroblast growth factor receptors. Effective treatments for HCC patients after progression or intolerance to sorafenib remain an unmet clinical need. This study was designed to assess brivanib in these patients.

Methods: This multinational, double-blind, randomized, placebo-controlled phase 3 study enrolled HCC patients whose disease had progressed on, or were intolerant to, sorafenib treatment. Patients stratified by study site, ECOG-PS, sorafenib outcome, and vascular invasion/extrahepatic spread were randomized (2:1) to receive brivanib 800 mg QD orally + best supportive care (BSC) (n=263) or placebo + BSC (n=132). The primary endpoint was OS. Key secondary endpoints were TTP, DCR, ORR and safety.

Results: Among 395 randomized patients, 87% had progres-

sion on sorafenib, 59% ECOG-PS 0, 72% vascular invasion/extrahepatic spread, 92% Child-Pugh A liver function, and 86% BCLC stage C. Baseline characteristics and prognostic factors were balanced between brivanib and the placebo, except for vascular invasion and AFP. Brivanib did not meet the primary endpoint of significantly improving OS vs the placebo (9.4 vs 8.2 mos; HR [95% CI], 0.89 [0.69–1.15]; $P=0.3307$), but showed improvement in TTP (4.2 vs 2.7 mos; HR, 0.56 [0.42–0.76]; $P=0.0001$), DCR (71.2% vs 49.1%; OR, 2.69 [1.65–4.38]; $P<0.0001$) and ORR (11.5% vs 1.9%; OR, 5.75 [1.40–23.62]; $P=0.0032$) based on the modified RECIST. Grade 3–4 AEs occurring at a higher rate ($\geq 5\%$) with brivanib vs the placebo were hypertension (19% vs 2%), hyponatremia (17% vs 7%), fatigue (15% vs 3%), decreased appetite (12% vs 2%), and diarrhea (9% vs 2%). Discontinuation due to treatment-related AEs was 23% for brivanib and 7% for placebo.

Conclusions: In this study, brivanib did not significantly improve the OS in advanced HCC patients in whom sorafenib treatment had failed. There were improvements in TTP, DCR and ORR, indicating brivanib anti-tumor activity. Brivanib had an acceptable safety profile.

Subcapsular Hepatocellular Carcinoma Can Be Safely Ablated through Indirect Puncture with Artificial Ascites: Analysis of 338 Cases of Radiofrequency Ablation for Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) located in the subcapsular area has a high risk of tumor seeding, incomplete ablation and adjacent organ injury. Creating artificial ascites could offer a better pathway for the radiofrequency ablation (RFA) electrode into the tumor with less chance of peritoneal seeding and thermal injury to adjacent organs. We aimed to evaluate the efficacy and safety of RFA for subcapsular HCCs with artificial ascites in a single center.

Patients and Method: We evaluated 338 treatment naïve patients diagnosed as having HCC and who underwent RFA from Jan 2005 to Dec 2008 in Kyungpook National University Hospital. The median age of the patients was 60.0 yr ranging from 24 yr to 88 yr. The male to female ratio was 251:87 (74.3%:25.7%). Subcapsular tumors were ablated with a Cool-tip electrode through an indirect puncture technique under artificial ascites. Patients were followed-up with abdominal computed tomogram and alpha-fetoprotein levels at one and then every 3 months after RFA.

Results: There were 169 cases (50%) of subcapsular tumors

and 169 tumors (50.0%) in the noncapsular area. The size of the tumors in each group was 1.9 ± 0.5 cm and 1.8 ± 0.6 cm, respectively. Incomplete ablation was observed in 10 patients (5.9%) in the subcapsular group and 5 in the nonsubcapsular group. There were 9 cases (2.7%) of treatment failure (6 cases in the subcapsular tumors, and 3 cases in the nonsubcapsular tumors). The cumulative disease free survival rates at 12, 24, and 36 months were 46.4%, 38.1% and 31.7%, respectively, in the subcapsular group compared to 71.8%, 41.1% and 29.4%, respectively, in the nonsubcapsular group ($p=0.132$). The time to progression was 10 ± 0.8 months in subcapsular group and 14.3 ± 0.2 months in the nonsubcapsular group ($p=0.249$). No significant differences were found in the overall survival (36 months; 63.4% vs 65.2%, $p>0.249$). There were 1 case of ileus in both groups and 1 case of pancreatitis in the subcapsular group.

Conclusions: RFA for subcapsular HCC can be safely performed with comparable treatment efficacy to that of nonsubcapsular HCC. Successful RFA in patients with subcapsular HCC can be performed by experienced operators with careful selection of patients and the use of artificial ascites.

CCRL1 Inhibits the Growth and Metastasis of Hepatocellular Carcinoma and Favors a Better Patient Survival

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Background: Atypical chemokine receptors are agonist-binding molecules that sequester inflammatory cytokines and signaling receptor components which are involved in cancer growth and metastasis. As a member of this group, Chemokine (CC-motif) receptor-like 1 (CCRL1) can mainly scavenge CCL19, CCL21 and CCL25. This study was aimed at investigating the expression and potential regulatory role of CCRL1 in human hepatocellular carcinoma (HCC).

Methods: We analyzed the expression of the CCRL1 by quantitative real-time polymerase chain reaction and Western blotting in HCC tissue and cell lines. The clinical significance of CCRL1 expression was analyzed using tissue microarrays (TMAs) containing 240 HCC cases. The effect of down-regulating CCRL1 on the proliferation and metastasis of HCC were investigated in vitro and in vivo.

Results: CCRL1 mRNA expression varied in HCC tumors and cell lines with a trend toward lower expression in a higher degree of malignancy. Immunostaining showed that significantly low CCRL1 expression was observed in tumors with poor differentiation ($P<0.01$) or an advanced TNM stage ($P<0.01$). Multivariate analysis indicated that patients in the CCRL1^{low} group had a significantly poorer outcome than those in the CCRL1^{high} group, in terms of overall survival ($P<0.05$) and cumulative recurrence

rates ($P<0.05$). CCRL1-knockdown increased the sequestration of CCL19 and CCL21 and hence enhanced proliferation and invasion of HCC cell lines in vitro ($P<0.05$), and increased tumor growth and lung metastasis in vivo ($P<0.05$). Furthermore, we found that down-regulation of CCRL1 could indirectly activate the protein kinase B (Akt) signaling pathway.

Conclusion: CCRL1 may restrict the growth and metastasis of HCC by scavenging CCL19 and CCL21 with the Akt signaling pathway involved. This finding may shed a light on a new therapeutic strategy for patients with HCC.

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Reduced Failure Rate of Hepatocellular Carcinoma Surveillance by Adopting Computed Tomography Or Magnetic Resonance Imaging as An Alternative Screening Method in Patients with Macronodular Cirrhosis

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Background & Aims: Initial presentation of hepatocellular carcinoma (HCC) at an advanced stage in patients under a regular surveillance program is a devastating problem. We assessed the prevalence and clinical factors associated with this surveillance failure.

Methods: A total of 304 patients who were diagnosed as having HCC under a regular surveillance program were retrospectively reviewed. Surveillance failure was defined when the tumor was diagnosed at beyond the Milan criteria. The surveillance programs consisted of radiological tests [ultrasonography (US) for the US-only surveillance (US-S) group ($n=48$, 15.8%); US with computed tomography or magnetic resonance imaging for the alternative surveillance (Alt-S) group ($n=256$, 84.2%)] and measurement of serum alpha-fetoprotein at 6 month intervals. We evaluated the factors associated with surveillance failure.

Results: Surveillance failure was noted in 18 of 304 patients (5.9%). Macronodular cirrhosis (MC) (yes vs. no: 10.3% vs. 3.2%; odds ratio (OR) 4.22; 95% confidence interval (CI): 1.28 – 13.90), US-S (yes vs. no: 14.6% vs. 4.3%; OR 3.59; 95% CI: 1.04 – 12.47), and infiltrative tumor type (yes vs. no: 57.1% vs. 3.4%; OR 35.38; 95% confidence interval CI: 9.09 – 138.33) were independent factors for surveillance failure. Based on the two baseline factors, the surveillance failure rates were 35.7%, 6.8%, 5.9% and 2.6% for MC (+)/US-S, MC (+)/Alt-S, MC (–)/US-S, and MC (–)/Alt-S, respectively ($p<0.001$).

Conclusions: The HCC surveillance failure rate was significantly higher for patients with MC who underwent US-S. Surveillance failure risk could be reduced by Alt-S in these patients.

The Efficacy of Transarterial Chemoembolization, Sorafenib, and Combination Therapy with Transarterial Chemoembolization and Sorafenib in Patients with Advanced Hepatocellular Carcinoma

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Background: Transarterial chemoembolization (TACE) and sorafenib have been approved as a standard therapy in intermediate stage and advanced hepatocellular carcinoma (HCC), respectively. The efficacy of TACE, sorafenib, and combination therapy with TACE and sorafenib in patients with advanced HCC was evaluated.

Methods: Between April 2002 and April 2012, a total of 107 patients with advanced HCC who were treated with TACE (n = 74), sorafenib (n = 15), and combination therapy with TACE and sorafenib (n = 18) were retrospectively enrolled. Patient characteristics, CT findings, TACE findings, and survivals were assessed. The Kaplan-Meier survival curve and Cox regression analysis were used to investigate the survival times and risk factors for mortality.

Results: The mean age of patients was 57.6 ± 10.6 years. The median overall survival time and 3, 6, and 12-month survival rates were 6.0 ± 1.8 months, 71%, 50%, and 42%, respectively. The median survival times of TACE, sorafenib, and combination therapy were 6.0 ± 1.8 , 5.0 ± 1.4 , and 22.0 ± 10.1 months, respectively. The 3, 6, and 12-month survival rates of each groups were 80%, 46%, and 37% (TACE therapy), 66%, 53%, and 53% (sorafenib therapy), and 89%, 71%, and 59% (combination therapy) (TACE vs. combination therapy: $p = 0.044$, sorafenib vs. combination therapy: $p = 0.048$, and TACE vs. sorafenib: $p = 0.554$). In the Cox regression analysis, Child-Pugh class C ($p = 0.007$, OR 2.839, CI 1.331–6.054) and combination therapy ($p = 0.028$, OR 0.397, CI 0.174–0.905) were related with mortality.

Conclusions: The combination therapy with TACE and sorafenib showed a survival benefit compared with TACE or sorafenib monotherapy in patients with advanced HCC. In addition, Child-Pugh class C was another risk factor of mortality.

HIF-2 α inhibits Hepatocellular Carcinoma Growth Arrest through The TFDP3/E2F1-dependent Apoptosis Pathway

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Background: Hypoxia inducible factors (HIFs) are activated in many tumors and show either promoter or suppressor activity depending on the tumor cell biology and background. However, the role of HIF-2 α , an HIFs member, remains unclear in hepatocellular carcinoma (HCC).

Methods: HIF-2 α expression was measured in HCC tissues with real time PCR, Western blotting and an immunofluorescence assay, and the clinical significance was detected with tissue microarrays obtained from 246 HCC patients. HIF-2 α expression levels were over-expressed or knocked-down using an expression or short hairpin RNA plasmid. Cells were analyzed with immunoblotting, chromatin immunoprecipitation coupled with microarray, co-immunoprecipitation, and histochemical staining. *In vivo* tumor growth was analyzed in nude mice.

Results: HIF-2 α was expressed unequally in HCC tissues and the decreased level was associated with lower overall survival ($p=0.006$). High HIF-2 α expression in HCC cells induced more pro-apoptosis protein expression and cell apoptosis, as well as being associated with inhibited cell and tumor growth. Furthermore, HIF-2 α inhibited the novel target gene transcription factor dimerization partner 3 (TFDP3) expression. TFDP3 bound with E2F1 and inhibited its transcriptional activity via both p53-dependent and independent pathways. Re-introduction of TFDP3 reversed HIF-2 α -induced apoptosis.

Conclusions: The data gathered from cell lines, the animal model and HCC patients showed a negative role of HIF-2 α , resulting from the TFDP3/E2F1 pathway. Our study uncovered another mechanism that linked HIF-2 α to a fundamental biologic regulator, E2F1 and provided clinical evidence elucidating the suppressor role of HIF-2 α .

Acute Interstitial Pneumonia Associated with Sorafenib Therapy in Advanced Hepatocellular Carcinoma Patients: Report of Three Cases

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Background: To date, several cases of interstitial lung injury caused by molecular targeting agents have been reported. Aim: To examine the clinical features of acute interstitial pneumonia (AIP) associated with sorafenib treatment.

Methods: We administrated sorafenib for 105 advanced hepatocellular carcinoma (HCC) patients, and from this group we investigated the clinical features of the patients who developed AIP.

Results: There were three cases of acute respiratory failure related with AIP that developed during sorafenib treatment in patients with advanced HCC. Case 1 was a 76-year-old male with lymph node metastases from HCC. Five days after the initiation of sorafenib, he developed dyspnea and fever. As chest X-ray and chest computed tomography (CT) scan showed interstitial pneumonia, we discontinued sorafenib treatment and initiated prednisolone therapy immediately, which was effective and the pneumonia improved in seven days. Case 2 was a 75-year-old male with recurrent HCCs after hepatectomy. Case 3 was a 77-year-old male with a huge sacrum bone metastasis from HCC. These two patients developed high grade fever and hypoxemia during sorafenib therapy, and were diagnosed as having AIP using chest CT scan, bronchoscopy and blood examination. Although we started high dose steroid therapy immediately, respiratory failure worsened and they died. In all of these three patients, serum KL-6 levels or surfactant protein D (SP-D) levels were elevated. Blood culture and sputum culture revealed no significant findings. Notably, all of them had been smokers and were asymptomatic before sorafenib treatment, although pretreatment respiratory examinations had shown restrictive lung impairment [the values of % vital capacity (%VC) was 47% in case 1, 76% in case 2 and 58% in case 3, respectively].

Discussion and Conclusion: The clinical features of our present three cases indicated that male, older age, smoking history and lung impairment before sorafenib therapy were closely associated with acute lung injury-related AIP during sorafenib treatment for advanced HCC.

Particle Radiotherapy Using Proton Or Carbon Ion Beams for Hepatocellular Carcinoma with Portal Vein Tumor Thrombus Extending to The Main Trunk or Major Branches.

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Background: The purpose of this study was to evaluate the clinical efficacy of particle radiotherapy using proton or carbon ion beams for hepatocellular carcinoma (HCC) with portal vein tumor thrombus.

Methods: Between April 2001 and December 2010, 63 patients with HCC having portal vein tumor thrombus extending to the main trunk (Vp4 group, 34 patients) or major branches (Vp3 group, 29 patients) were treated with particle radiotherapy using a proton (49 patients) or carbon ion (14 patients) beams. Of all patients, 42 patients had multiple intrahepatic metastases (IM). The median tumor size was 65 mm (range, 30–140 mm).

Results: The overall survival (OS) rates of all patients for the 1 year, 2 year, and 3 year were 48%, 22%, and 13%, respectively. The OS rates of the patients without IM (14 patients) among the Vp3 group for 1 year, 2 year, and 3 year were 75%, 48%, and 32%, and those with IM (15 patients) among the Vp3 group were 38%, 0%, and 0%, respectively. Those without IM (7 patients) among the Vp4 group were 80%, 60%, and 20%, and those with IM (27 patients) of Vp4 group were 28%, 0%, and 0%, respectively. Multi-variable analysis revealed that a factor with or without IM significantly influenced OS. All acute toxicities were transient. Grade 3 late toxicities of liver damage according to Common Terminology Criteria for Adverse Events v3.0. were observed in 3 patients.

Conclusions: Particle radiotherapy produced favorable results as treatment specifically for Vp3 without IM, and may be a novel treatment for HCC with portal vein tumor thrombus.

The Establishment of an Optimized Geometrical Mathematical Model of Radiofrequency Ablation in Hepatic Carcinoma

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Radiofrequency ablation (RFA) is an effective means of achieving local control of liver cancer. It is effective for small and favorably situated tumors, but local progression rates are substantially higher for large tumors (>3 cm). In this paper, a mathematical model based on geometrical optimization was established

to treat large liver tumors. A database of mathematical models regarding the configuration of liver cancer was also established. The electrode placement and frequency of ablation were also optimized. Meanwhile, three types of liver cancer lesions were simulated on a computer guided with the mathematical model. This method was expected to provide a more effective and intelligent way for RFA in hepatic carcinomas.

Adenovirus-Mediated MiRNA against Human Fibrinogen like Protein 2 Inhibits Hepatocellular Carcinoma Growth

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Aims: As a member of the fibrinogen-related protein superfamily, Fibrinogen-like protein 2 (also called fgl2 prothrombinase) has serine protease activity and directly catalyzes prothrombinase into activated thrombinase, which is considered as an important link between tumor growth and metastasis of human cancer cells. We reported that human fgl2 (hfgl2) was overexpressed in various human solid tumor tissues and associated with the tumor growth, invasion and angiogenesis of a HCCLM6 hepatoma cell line. In the present study, an adenovirus vector expressing miRNA against hfgl2 gene was used to evaluate the effect on HCCLM6 cell proliferation and growth of an HCCLM6 tumor xenograft in nude mice.

Methods: We constructed a replication-deficient recombinant adenovirus which encoded a miRNA specifically targeting hfgl2 (Ad-hfgl2-miRNA). Its effect on target gene expression and HCCLM6 cells proliferation was analyzed with reverse transcription quantitative polymerase chain reaction (RT-QPCR), Western blotting and flow cytometry. The xenograft tumor model in nude mice was established by subcutaneous inoculation with 5×10^6 HCCLM6 cells, and the effect of gene targeting was estimated with Ad-hfgl2-miRNA intratumorally injected when the tumor volume reached 100 mm³. The volume of the tumor xenograft was recorded twice a week and the relative tumor volume (RTV) was calculated. The levels of hfgl2 mRNA, protein and angiogenesis in tumor xenograft were detected with RT-QPCR and immunohistochemical staining.

Results: Ad-hfgl2-miRNA was constructed successfully, which downregulated hfgl2 expression significantly on both mRNA and protein levels in a sequence-specific manner and exerted a proliferation inhibitory effect on HCCLM6 cells (proliferation index decreased from 2.36 in the irrelevant group to 1.74 in the interference group, $P < 0.05$). In the HCCLM6 tumor xenograft established in nude mice, the tumor volume significantly decreased following the intratumoral injection of Ad-hfgl2-miRNA as compared with those of the irrelevant plasmid and blank con-

trol groups (RTV reached 25.66% in interference group, $P < 0.05$). In addition, Ad-hfgl2-miRNA also exhibited potent antiangiogenic effects.

Conclusion: These data suggest that hfgl2 might be a potential target for an antiangiogenic effect in hepatocellular carcinoma. Hfgl2 gene silencing using miRNA may be an alternative strategy for management of HCC.

Lipocalin-2 Negatively Modulates Twist-1-mediated Epithelial-mesenchymal Transition in Hepatocellular Carcinoma

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Purpose: Our purpose in this study was to investigate whether Lcn2 is involved in hepatocellular carcinoma (HCC) development via its involvement in invasion and metastasis.

Experimental Design: Lipocalin-2 (Lcn2) expression was investigated with DNA microarray technology in HCC tissue samples, and its expression was validated in human HCC tissues and cells. The changes in proliferation, migration, and invasion ability by stable expression or knockdown of Lcn2 were measured in HCC cells. Tumor growing and metastasis modulated by Lcn2 were tested in an in vivo mouse model.

Results: Using unsupervised hierarchical clustering analysis of tissue samples, we identified preferential expression of Lcn2 (>2-fold change) in the differentiated HCC versus the liver cirrhosis tissues. The characteristics of epithelial-mesenchymal transition (EMT) were reversed by adenoviral transduction of Lcn2 into SH-J1 cells, including the down-regulations of N-cadherin, vimentin (VIM), alpha-smooth muscle actin (alpha-SMA), and fibronectin (FN), and the concomitant up-regulation of CK-8, CK-18, and desmoplakin I/II (DesI/II). Knock-down of Lcn2 by shRNA in HKK-2 cells was associated with EMT change. EGF or TGF- β treatment resulted in downregulation of Lcn2 with a similar EMT change. Stable Lcn2 expression in HCC cells reduced the expression of the transcription factor Twist1, resulting in the inhibition of cell proliferation, migration, and invasion in vitro, and suppressed tumor growth and metastatic ability in a mouse model.

Conclusions: These findings suggest that Lcn2 can reverse the EMT in HCC cells through transcriptional suppression of Twist 1. Thus, Lcn2 may be a candidate metastasis suppressor and a potential therapeutic target for HCC.

Clinical Safety and Tumor Response Rate of Hepatocellular Carcinoma after TACE Using Drug-Eluting Beads

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Background: To assess the tumor response rate of hepatocellular carcinoma (HCC) after transarterial chemoembolization using drug-eluting beads (DEB-TACE).

Materials and Methods: This study enrolled 129 patients with HCC who had undergone DEB-TACE since September 2009. The liver function status of all patients was either Child-Pugh class A (n=112) or B (n=17), and BCLC stages were 0 (n=20), A (n=57), B (n=34) and C (n=18). The size of the DEB was either 100–300 or 300–500 µm, and the maximum loading dose of the chemotherapeutic agent was 140 mg of doxorubicin. The procedure end-point was stasis of arterial inflow of the feeding arteries for at least 10 seconds on 10 minutes delayed angiography as well as complete disappearance of tumor staining. The tumor response rate was assessed according to the EASL criteria.

Results: There were no major complications except mild transient post-embolization syndrome. Only five (3.9%) patients showed deterioration of their Child-Pugh score. Until the last follow-up period (1–24 months), under the EASL criteria 64.8% were rated as CR, 20.4% as PR, 6.5% as SD and 8.3% as PD (85.2% of OR).

Conclusion: DEB-TACE was considered as a safe modality for the treatment of HCC, and showed good a tumor response rate in this mid-term result.

Identification of The DSCC1, a Subunit of an Alternative RFC Complex, as a Candidate Oncogene in Hepatocellular Carcinoma

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Background and Aims: DSCC1 located at 8q24 and is frequently amplified in hepatocellular carcinoma (HCC). It has not been fully understood, however, how DSCC1 is involved in the carcinogenesis and progress of HCC. The aim of the present study was to examine the importance of DSCC1 in the evolution and progression of HCC.

Method: The copy number variation and expression pattern of DSCC1 in HCC tumor and cell lines were analyzed with a bioinformatics analysis and validated with a QRT-PCR analysis.

DSCC1 was knocked down in HCC cell lines using a lentivirus mediated shRNA. The impact of DSCC1 silence on the in vivo clonogenic growth of HCC cell lines was used to determine its importance in HCC. Flag tagged DSCC1 was used to rescue the phenotypic changes caused by the depletion of DSCC1. Co-Immunoprecipitation (Co-IP) followed by mass spectrometry was used to identify the proteins which interacted with DSCC1 in the MHCC-97H cell lines.

Results: DSCC1 was not detectable in normal livers. On the other hand, 17% (18/103) of HCC patients had a more than two-fold gain in the DSCC1 coding region, and 65% (243/374) of HCC patients had an increase in DSCC1 mRNA levels in primary tumor tissues as shown with the microarray data analysis. In our cohort, DSCC1 was significantly overexpressed in primary tumor tissues of 76% (23/30) of HCC cases. Six of the seven analyzed HCC cell lines overexpressed DSCC1, among which were MHCC-97H, MHCC-97I and Hep3B carrying 8q24 amplification. Strikingly, the clonogenic growth potential of each of the three DSCC1-amplified cell lines, but none of the nonamplified cell lines, was significantly reduced by silencing of DSCC1, which suggested that HCC cell lines carrying DSCC1-amplification showed oncogene dependence on DSCC1. Re-expression of DSCC1 in these DSCC1-depleted cell lines successfully rescued their clonogenic ability. Furthermore, DSCC1 was found to interact with CTF8, CTF18, RFC2 and PCNA in an MHCC-97H cell line.

Conclusion: DSCC1 is a candidate oncogene in HCC and a possible HCC treatment target. The mechanism of DSCC1 in the carcinogenesis of HCC may involves the RFC complex and the regulation of PCNA.

Matched Pair Analysis Comparing the Clinical Outcome of Hypofractionated Radiotherapy with tomotherapy and 3-dimensional Conventional Radiotherapy in Locally Advanced Hepatocellular Carcinoma

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Introduction: To investigate whether hypofractionated radiotherapy (RT) with tomotherapy could improve the clinical results for locally advanced hepatocellular carcinoma (LAHCC).

Methods: In this retrospective-matched cohort study, 53 patients were included who had undergone hypofractionated RT with tomotherapy and 53 patients with 3DCRT using a linear accelerator, treated between 1992 and 2010. The two groups were matched 1:1 for 7 potential prognostic factors including age, gender, tumor size, T category, N category, TNM stage, and liver cirrhosis and were evaluated for overall survival (OS) and progression free survival (PFS). To account for the matched pair analysis,

we used the Cox regression model to estimate the hazard ratios. Kaplan-Meier survival analyses were used to estimate the OS and PFS. We used the log-rank test and Breslow test to estimate the statistical significance of survival differences.

Results: Since the mean values of the biologically effective dose (BED, α/β ratio=10) were 63.28 Gy in the hypofractionated RT group and 55.18 Gy in the 3DCRT group, hypofractionated RT with tomotherapy could deliver a significantly higher BED than 3DCRT ($p<0.0001$). The median follow-up periods for surviving patients after RT were 25.93 months in the hypofractionated RT group and 38.3 months in the 3DCRT group. The median survival rates were 19.77 months and 12.87 months for the hypofractionated RT and 3DCRT groups, respectively. The 1-year, 2-year, and 3-year OS rates were 81.1%, 42.2% and 31.6% in the hypofractionated RT group and 52.8%, 30.2% and 25.9% in the 3DCRT group, respectively ($p=0.073$ in log rank test, $p=0.009$ in Breslow test). The 1-year, 2-year, and 3-year PFS rates were 50.9%, 29.8% and 18.5% in the hypofractionated RT group and 40.9%, 28.7% and 17.9% in the 3DCRT group, respectively ($p=0.1$ in log rank test, $p=0.024$ in Breslow test).

Conclusion: This study showed an OS and PFS benefit of hypofractionated RT with tomotherapy in the treatment of LAHCC. Hypofractionated RT with tomotherapy seems to improve the clinical outcome for LAHCC patients.

achieved a partial response. Local failure, intrahepatic recurrence, and distant metastasis were observed in 7 (7.5%), 58 (62.4%), and 20 (21.5%), respectively. Cumulative incidence of local recurrence rates at 1 and 3 years were 5.2% and 7.9% respectively. Overall survival rates at 1 and 3 years were 86.0% and 53.8%, respectively. Grade ≥ 2 hepatic toxicity was observed in 18 (19.4%) and progression of the Child-Pugh score by 2 or more developed in 9 (9.7%) patients.

Conclusion: SBRT for HCC appears to be a safe and promising non-invasive treatment modality for the local management of this tumor. Further study of combinations of SBRT and other modalities may be desirable because progressive disease outside the treated area is the main cause of failure.

Multicenter Validation Study of The Prognostic Index for Portal Vein Tumor Thrombosis in Patients with Hepatocellular Carcinoma Treated with Radiation Therapy: Comparison among The Okuda, CLIP, CUPI and JIS Staging Systems (KROG 11-05)

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Background: Our group previously reported a staging system known as the PITH, prognostic index for portal vein tumor thrombosis (PVTT) in hepatocellular carcinoma (HCC) patients when treating with radiation therapy (RT)¹. The PITH score was defined as the number of the following factors: ECOG performance status, Child-Pugh class, multiple tumors, main PVTT, lymph node metastasis, and primary tumor size. This study aimed to validate the PITH staging system and compare the predictive power with

Stereotactic Body Radiation Therapy for Primary or Recurrent Hepatocellular Carcinoma: Long-term Patient Outcomes.

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Background: Stereotactic body radiation therapy (SBRT) offers a treatment option for hepatocellular carcinoma (HCC) patients with small tumors who are not eligible for surgery or radiofrequency ablation. In this analysis, we evaluate the long-term clinical results of SBRT for small, unresectable HCCs.

Methods: A registry database of 93 patients (103 lesions) treated with SBRT for primary or recurrent HCC between March 2007 and December 2009 was analyzed retrospectively. A dose of 10–20 Gy (median, 15 Gy) per fraction was given over 3–4 consecutive days to a total dose of 30–60 Gy (median, 45 Gy). The tumor response was determined by computed tomography or magnetic resonance images performed 3 months after completion of SBRT. Radiation-induced hepatic toxicity was graded according to the Common Terminology Criteria for Adverse Events or a decline in liver function using the Child-Pugh score.

Results: The median follow-up was 25.6 months. We found that 51.5% of patients achieved a complete response and 21.4%

other published staging systems in those patients.

Methods: We performed a retrospective study, and 994 HCC patients with PVTT treated with RT between 1998 and 2011 from the Korean Radiation Oncology Group were analyzed. All patients were staged with the PITH, Cancer of the Liver Italian Program score (CLIP)², Japanese Integrated Staging (JIS)³, and Okuda systems⁴ before RT and the survival data were analyzed. The likelihood ratio test was performed to measure homogeneity and the Akaike information criteria (AIC) were used to evaluate the discriminatory ability of the given staging systems.

Results: The median survival was 9.2 months. All staging systems including the PITH reached statistically significant survival differences but generally PITH showed a better stratification ability. The likelihood ratio test within a Cox proportional hazard regression model showed the PITH to have a higher Chi-square (1238.053) than the others (Okuda 45.893, CLIP 89.056, JIS 78.595). Furthermore, a lower Akaike information criteria value was shown in the JIS score (9977.135) than in the others (Okuda 10022.270, CLIP 9980.722, JIS 9987.870), proving that the PITH staging system was a better prognostic model.

Conclusion: The predictive power of the PITH staging system was better than the other staging systems in HCC patients with PVTT treated with RT.

Gene Polymorphism and mRNA Levels of Cytochrome *P450IIE1* and *GSTP1* in Patients of Different Nationalities with Alcoholic Liver Disease (ALD)

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Aim: To investigate, in patients of three nationalities, the relationship between alcoholic liver disease and the genetic polymorphism and expression levels of two enzymes, cytochrome *P450IIE1* and the *GSTP1* glutathione S-transferase.

Methods: Peripheral blood was collected from 353 Chinese patients with alcoholic liver disease, 300 alcohol-dependent patients without liver disease, and 360 healthy controls. Each group included patients from the Han, Chaoxian, and Mongol nationalities. Real-time polymerase chain reaction (RT-PCR) was used to measure the level of *P450IIE1* and *GSTP1* mRNA, and PCR restriction fragment length polymorphism (PCR-RFLP) was used to detect which polymorphic alleles of each enzyme were carried by each patient.

Results: Regardless of nationality, patients who carried the rare *CYP11E1* C2 and *GSTP1* Val alleles were at higher risk of ALD. The frequency of C2 and Val in patients with ALD was, respectively, 50.00% and 26.98% among the Han, 31.36% and 22.87% in Mongol patients, and 45.87% and 22.02% in the Chaoxian. In comparison, the frequency of the C2 and Val genotypes in the con-

trol group were, respectively, 10.00% and 8.3% for the Han nationality, 7.50% and 10.00% in Mongols, and 11.67% and 9.17% in the Chaoxian. No significant differences were seen in the frequency of either the C2 or Val alleles, or in the frequency of ALD, among the three nationalities ($p > 0.05$). In each nationality, the frequency of both the C2 and Val alleles was significantly higher in ALD compared to control patients ($p < 0.01$). Regardless of nationality, the mRNA level of *CYP11E1* was higher, and that of *GSTP1* lower, in patients with ALD compared to controls.

Conclusion: In this group of Chinese patients, we saw, regardless of nationality, that patients with ALD tended to have a higher expression of *CYP11E1* and to carry the C2 allele, and tended to have a lower expression of *GSTP1* and to carry the Val allele. This may indicate a causal relationship between these polymorphic alleles and the development of ALD.

Immunosuppressive Effects of Sorafenib on Natural Killer Cells in Murine Models with Or without Hepatocellular Carcinoma

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Background: Sorafenib, a multi-tyrosine kinase inhibitor, has proven its therapeutic efficacy in advanced hepatocellular carcinoma (HCC). However, little is known about its effects on the immune system. In this report, we examined the effects of sorafenib on the natural killer (NK) cells in murine models with or without HCC.

Methods: The orthotopic models of HCC in C57/BL6 mice were established with Hepa1-6 cells. The ratio of NK cells and the expression of CD69 on NK cells were examined when treated with sorafenib (30 mg/kg and 60 mg/kg daily, two weeks) or a vehicle as the control in tumor-bearing mice and non-tumor-bearing mice. NK cells in treated murine spleens were separated with a MACS assay and the cytotoxicity of NK cells against Yac-1 cells was examined with an LDH release assay. Lung metastatic colonies were detected four weeks after intravenous incubation of HepG₂-GFP cells in nude mice pretreated with sorafenib (60 mg/kg daily, two weeks) or a vehicle.

Results: Sorafenib treatment significantly reduced the ratios of the NK cells in spleens in a dose-dependent manner both in tumor-bearing mice and in non-tumor-bearing mice. The ratios of NK cells in spleens from the control, sorafenib 30 mg/kg/day, and 60 mg/kg/day were $3.48 \pm 0.32\%$, $2.38 \pm 0.16\%$, and $2.13 \pm 0.17\%$, respectively, in tumor-bearing mice, and $2.73 \pm 0.28\%$, $2.03 \pm 0.21\%$, and $1.83 \pm 0.16\%$ in non-tumor-bearing mice. However, the change in CD4⁺ T cells and CD8⁺ T cells was not obvious. Sorafenib also down-regulated the stimulatory receptor CD69 on NK cells in tumor-bearing mice, but not in non-tumor-bearing mice. Sorafenib also inhibited the cytotoxicity against Yac-1 cells both in tumor-

bearing and no-tumor-bearing-mice. Sorafenib pretreatment could also promote lung metastasis. The ratios of lung metastatic area to the total area of lung in mice pretreated with sorafenib and the vehicle were $17.4 \pm 1.15\%$ and $8.2 \pm 1.25\%$ ($p < 0.01$).

Conclusions: Pretreatment with sorafenib promoted lung metastasis and sorafenib treatment exhibited immunosuppressive properties, which may be attributed to the decrease in number and activity of NK cells. For patients with HCC, immunotherapeutic approaches could potentially enhance the effects of sorafenib when given in combination with sorafenib.

SIRT1 Displays Anticancer Potential against Hepatocellular Carcinoma with Loss of P53 Function

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Background: Hepatocellular carcinoma (HCC) is a highly malignant tumor with a poor prognosis. SIRT1 (silent mating-type information regulation 2 homologue 1) is a class III histone deacetylase that is implicated in gene regulation and stress resistance. Detection of SIRT1 is considered a useful predictor of the prognosis in some carcinomas. However, its interpretation and clinical significance remain to be determined in HCC.

Methods: A retrospective study was performed on the clinicopathological features of HCC from surgical cases. Protein expression level was detected by Western blotting. p53 mutation was identified by sequencing.

Results: In this study, we found that SIRT1 was a strong and independent predictor for better prognosis in HCC patients with loss of p53 function. Down regulation of SIRT1 by its inhibitor EX527, siRNA or shRNA consistently stimulated and reduced the cell growth and proliferation of Hep3B cells (loss of p53 function) and HepG2 cells (gain of p53 function) respectively in vitro or *in vivo*. SIRT1 inhibition or silencing caused suppression of AMP-activated protein kinase (AMPK) activity and subsequently enhanced mammalian target of rapamycin (mTOR) activity, resulting in induction of S6 Kinase 1 (S6K1) activity in Hep3B but not HepG2 cells in vitro and *in vivo*. Consistently, we detected a significant positive correlation between SIRT1 and phosphor-AMPK expression levels in a cohort of HCC specimens with loss of p53 function, and the combination of these two parameters was a powerful predictor of the prognosis in HCC patients with loss of p53 function.

Conclusions: SIRT1 displays an anti-carcinogenic role through the AMPK-mTOR pathway in HCC with loss of p53 function. However, in HCC with normal p53, SIRT1 plays its role as a tumor promoter through its classical p53 pathway. The decision of the role following SIRT1 activation is greatly influenced by the loss or normality of p53 function.