Oral Presentation II

II-1

What Is the Best Strategy of Immunosuppression and Anti-Tumor Drug for Patients with Hepatocellular Carcinoma after Liver Transplantation?

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Purpose: We usually use rapamycin for patients with HCC after liver transplantation because of the anti-tumor effect of rapamycin. However, rapamycin only immunosuppression is sometimes not enough in terms of immunosuppression and anti-tumori effect after transplantation. We investigated the anti-tumoric efficiency of rapamycin only immunosuppression and rapamycin combined with other immunosuppressant and finally, rapamycin combined with anti-tumor drug including cisplatin or sorafenib or 5-FU.

Materials and Methods: We studied anti-tumoric effects of various combinations of immunosuppressants in three HCC cell lines (HepG2, Hep3B, Huh7). We used single regimen such as rapamycin only or tacrolimus only or MMF only regimen and then, we tried rapamycin with tacrolimus or rapamycin with MMF regimen. And then, we added anti-tumor drug such as sorafenib or cisplatin or 5-FU to previous combinations. To elucidate the underlying molecular signaling pathway, we performed Western blotting for phosphorylated p70 S6 kinase protein expression. We used immunosuppressants including rapamycin (5 ng/ml), tacrolimus (5 ng/ml), MMF (500 ng/ml) and anti-tumor drugs including cisplatin (5 uM/L), 5-FU (200 ng/ml), sorafenib (250 uM/L). We performed MTT assay (24 well) after incubation according to previously reported protocol.

Results: Tumor growth after treatment of rapamycin or tacrolimus or MMF were at the level of 64.9%, 98.5% and 69.4% respectively compared to no-treatment control group. Immunosuppression with rapamycin combined with MMF inhibited tumor cell growth at the level of 59.9% comparing to control group in Huh7 cell lines and more effective than rapamycin only treatment (p=0.0287). Treatment with rapamycin combined with MMF and sorafenib also inhibited tumor cell growth (53.2%) more effectively than immunosuppressant with other anti-tumor drug in Huh7 cell lines. These results were similar in other cell lines- HepG2 and Hep3B.

Conclusions: Rapamycin is often used as immunosuppressant for patients with high risk of tumor recurrence after liver transplantation but rapamycin only immunosuppression is sometimes not enough to prevent rejection and tumor recurrence. We should add another immunosuppressants or anti-tumor drug including tacrolimus, MMF or steroid and sorafenib, cisplatin or 5-FU. This study shows that the regimen of rapamycin combined with MMF may have better anti-tumor effect than rapamycin only treatment and cisplatin with rapamycin and MMF can be also effective anti-tumor treatment for patients with hepatocellular carcinoma after liver transplantation.

II-2

Identification of Clinical Factors That Affect Yield of Human Hepatocyte Isolation for Primary Culture

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Purpose: Primary hepatocyte culture is a challenging but an important issue that can potentially open numerous possibilities for basic research and clinical applications. Most of the studies have been undertaken using animal models and have made significant progress in the methodology of culture. But very few studies have been performed using human liver tissue so far. In preparation of primary hepatocyte culture using human liver tissue, clinical factors that influence isolation of viable human hepatocytes were investigated.

Materials and Methods: Fresh liver tissues were provided by 36 volunteered donor undergoing liver resection for various reasons. Hepatocytes were dis-

sociated using 1% collagenase solution per protocol. Isolated hepatocytes were stained with trypan blue for viability assessment and viability was calculated. Clinical parameters were collected by review of charts and pathologic slides. Multiple linear regression was performed using SPSS 12.

Results: Six patients had benign diseases, and 30 patients had malignant diseases. Among many clinical variables, age of donor (p=0.11), underlying malignancy (p=0.012), presence of cirrhosis ($p<0.001$), hemoglobin level (p=0.004), platelet number ($p<0.001$), protein level (p=0.005), albumin level (p=0.011), aspartate aminotransferase(AST) (p=0.026), Prothrombin time (INR) (p=0.006), Indocyanine blue 15 minute retention rate ($p<0.001$), preoperative chemotherapy (p=0.002) were statistically significant factors related with hepatocyte yield.

Conclusions: Yield of viable human hepatocytes in preparation of primary culture was influenced by many clinical factors that are important in evaluation of underlying liver function. Underlying malignancy, preoperative chemotherapy, and nutritional status were also significant. Preoperative patient information can give valuable basis for viable hepatocyte isolation.

II-3

Preoperative Prediction of Aggressiveness in Early-stage Hepatocellular Carcinoma for Safe Local Ablation Therapy

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Purpose: Radiofrequency ablation (RFA) has been reported to show comparable outcome with surgery for the treatment of hepatocellular carcinoma(HCC) with 3 or fewer tumors of up to 3 cm (early-stage HCC). However, satellite nodule can be present in early-staged HCC and results in local recurrence after local therapy. Poor tumor grade (Edmonson and Steiner's histologic grade $\geq$ 3) or microvascular invasion were known as risk factor for satellite nodules. Therefore, if the risk of satellite nodules is high, surgery is recommended than local therapy. The aim of the present study was to clarify the preoperative predictors of aggressiveness in early-stage HCC for safe local ablation therapy.

Materials and Methods: Among 1,086 patients underwent a hepatic resection in Seoul National University Hospital from January 2006 to December 2010, 152 patients with newly diagnosed early-stage HCC who underwent hepatic resection were enrolled in this study. Potential preoperative factors (AFP, PIVKAII, combined AFP and PIVKA, platelet, PT-INR, hepatitis B surface antigen, hepatitis C virus antibody and indocyanin green retention rate at 15 min) were investigated to elucidate the predictive factors for the microvascular invasion and poor histologic grade.

Results: Poor histologic grade was found in 28.3% and microvascular invasion was found in 16.4% of study cohort. Combined AFP and PIVKAII (AFP*PIVKA) >1,600 was a significant risk factor for poor histologic grade in univariate and multivariate analysis (16.4% vs 40.7%, $p<0.001$). Furthermore, combined AFP and PIVKA was significant risk factor for microvascular invasion in the univariate analysis (p=0.048). No factor was found significant in multivariate analysis.

Conclusions: Combined AFP and PIVKA is an useful predictors of the aggressiveness in the early-stage HCC. If the level of this predictor is high in the early-stage HCC, anatomic surgical resection should be recommended than local ablation therapy.

II-4

The Prognostic Significance of the Worst Grade in Hepatocellular Carcinoma with Mixed Histologic Grades

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Backgrounds and Aims: The tumor differentiation

has been known to one of the prognostic factors after the treatment of hepatocellular carcinoma (HCC). According to the 7th edition of the AJCC cancer staging manual, the grading scheme of Edmondson and Steiner (ES) is recommended. In HCC, multistep carcinogenesis frequently leads to mixed histologic grades. However, there has been no study about the prognostic significance of the worst grade in HCC with mixed histologic grades. The current study attempted to reveal which determines the prognosis after resection, the major or the worst grade in mixed histologic types.

Materials and Methods: From January 1996 to March 2010, a total of 724 patients underwent curative resection of HCC at Yonsei University Health System, Korea. Among them, 99 who had total necrosis due to previous treatment were excluded. Six hundred and twenty-five patients were first classified into homogenous and mixed grades. HCC with homogenous grade was further divided into three groups: HG1 (ES I, n=16), HG2 (ES II, n=241) and HG3 (ES III, n=156). Mixed histologic group was classified into M1 (n=52) and M2 (n=142) which had the worst histologic grade ES II and ES III, respectively. Disease-free survival (DFS) and overall survival (OVS) in each group were analyzed, and clinicopathologic features between each group were compared.

Results: 5-year DFS and OVS in each group were as follows: HG1 is 52.8% and 87.5%, M1 is 52.5% and 83.2%, HG2 is 52.2% and 71.4%, M2 is 43.5% and 55.1%, HG3 is 38.5% and 52.8, respectively. No statistically significant difference in survival was observed among HG1, M1, and HG2. However, the rates of DFS and OVS were significantly lower in M2 compared with HG2 (P=0.004 and 0.025, respectively) whereas DFS and OVS of M2 were not significantly different from HG3. There were no significant differences in the clinicopathological features of HG2, M2 and HG3 except that microvascular invasion was more frequently observed in M2 than HG2. In multivariate analysis, more advanced histologic group (M2 and HG3) was one of independent poor prognostic factors for DFS and OVS after curative resection. (P=0.028 and $<$0.001; relative risk, 1.367 and 1.769, respectively).

Conclusions: In this study, HCC with the worst grade ES III showed similar clinicopathologic characteristics and prognosis compared with HCC with homogenous ES III. Therefore, in patients with advanced histologic grade ($\geq$ES II), the worst histologic grade may determine the prognosis after curative resection of HCC.

II-5

The Results and Usefulness of Protocol Liver Biopsy in Patient with Normal Graft Function Who Underwent Liver Transplantation due to Hepatitis C Virus Related Liver Disease

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Purpose: Hepatitis C virus (HCV) recurrence after liver transplantation (LT) is universal and progressive. Serial liver biopsies remain the best way of monitoring disease progression. However, little is known about the results of protocol (nonevent-driven) biopsy in patient with normal graft function after LT regardless of clinical significance, especially in Korea. The aim of this study was to investigate the results of protocol liver biopsy in patient with normal graft function who underwent LT due to HCV related liver disease.

Materials and Methods: We have performed a protocol liver biopsy at specific time points (3 month, 6 month and 1 year after LT) in patient who underwent LT due to HCV related liver disease at Seoul National University Hospital in Korea since 2010. In this study, we retrospectively reviewed the results of protocol biopsy in HCV related liver transplant recipients between January 2010 and December 2011.

Results: Total 30 patients (8.9%) underwent LT due to HCV related liver disease among 337 recipients for 2 years at our center. Among them, 10 patients (33.3%, 10/30) with clinically normal graft function and almost normal liver function test underwent protocol biopsy within a year after LT. Seven patients underwent living donor LT and 3 patients deceased donor LT. Four patients had a coexisting hepatocellular carcinoma. No significant complication after the sono-guided needle biopsy was reported. With the

specific time points of biopsy, 6 patients underwent protocol biopsy at 3 month, 7 patients at 6 month and 3 patients at 1 year after LT, respectively. After protocol biopsy, we treated HCV infection in case of presence of fibrosis or in case of inflammation more than moderate degree based on the biopsy results. Finally, the treatment for HCV infection was started in 1 patient (16.7%, 1/6; due to portal fibrosis) after the protocol biopsy at 3 month, 3 patients (42.9%, 3/7; due to 2 periportal fibrosis, 1 moderate inflammation) at 6 month, 1 patient (33.3%, 1/3; due to septal fibrosis) at 1 year following LT. As the results, even clinical and laboratory liver function is normal, a significant portion of protocol biopsies within a year after LT show histologic abnormalities which require treatment for HCV recurrence following LT.

Conclusions: In HCV related liver transplant recipients, protocol liver biopsy within a year after LT provides important histological information and those abnormal findings despite the normal graft function can lead to early treatment of HCV recurrence following LT. Therefore, this may contribute to improve the survival in HCV related liver transplant recipients.

II-6

Model for End-Stage Liver Disease (MELD) Reflects Short Term Mortality of the Patients who Registered to Waiting List for Cadaveric Donor Liver Transplantation Better than CTP Based System

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Purpose: We have been used CTP score based allocation system in Korea although Meld system is better than CTP score based system in terms of objectiveness. There are many reports that show MELD system is more appropriate in predicting short term survival and mortality. Recently, there is a movement to change the allocation system to MELD score based system. We should collect the data and analyze the survival of the patients of waiting list without liver transplantation for the concensus to change the system.

Materials and Methods: The retrospective data of 453 patients who had registered to waiting list for cadaveric donor liver transplantation from January 2008 to May 2011 in Seoul National University Hospital were collected. We excluded patients whose age less than 12 and patients who underwent liver transplantation after registration. We analyzed the survival rates after registration according to new scoring system and current system. We performed ROC curve analysis for cut-off value of MELD score for 1 month mortality.

Results: Among 453 patients, we divided patients into two groups as high risk of mortality group including status 1, status 2A, status 2B and relative low risk group such as status 3 and status 7. Total number of high risk group patients was156 and total number of low risk group patients was 297. 1 Month mortality of low risk group was 8.3% and that of high risk group was 17.8%. 1 month mortality rates of even status 1 patients(n=9) was just 44.4%(n=4). 1, 3, 6 month mortality rates of status 2A patients(n=37) was 27%, 67% and 75.7% respectively and 73% patients survived after 1 Month. In status 2A patients group, mortality rates increased sharply about 40.9% when the MELD score was more than 30.

Conclusions: On the basis of the data of SNUH waiting list, MELD score based system reflects the short term patients survival or mortality better than CTP score based system. And it is more objective because Meld score system uses just lab data for scoring. In terms of definition of status 2A, current system can overestimate the condition of the patients. The elevation to status 2A of patients whose MELD score is low shoud be delayed until 1 or 2 month according to this study.