論　文　内　容　要　旨

Significant Association Between *FOXP3* Gene Polymorphism and Steroid-Resistant Acute Rejection in Living Donor Liver Transplantation

（成人間生体肝臓移植において*FOXP3*遺伝子多型とステロイド抵抗性拒絶反応には有意な相関がある）

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Regulatory T cells (Tregs) expressing the transcription factor forkhead box P3 (Foxp3) are essential for immune homeostasis. The development and function of Tregs is controlled by the *FOXP3* gene, encoding Foxp3 protein, which regulates T cell activation and functions as a transcriptional repressor to downregulate cytokine production in T cells. Polymorphisms in the promoter region of *FOXP3* may potentially alter gene expression by changing the binding specificity of transcription factors to their binding sites and by modifying the kinetics of transcription initiation, causing Treg dysfunction and consequently, the development of autoimmune diseases. Because preferential accumulation of Tregs in liver allografts during acute cellular rejection (ACR) is reportedly associated with reduced severity of rejection, suggesting a role of Tregs in preventing excessive progress of ACR, the present study investigated the impact of *FOXP3* SNPs on the severity of ACR in liver transplant (LT) recipients. A total of 102 living donor LT patients were enrolled in this study and categorized into no rejection, steroid sensitive acute rejection (SSAR) and steroid resistant acute rejection (SRAR). Genotyping of *FOXP3* SNPs -3499 A/G (rs3761547), -3279 A/C (rs3761548) and -924 A/G (rs2232365) was performed using polymerase chain reaction restriction fragment length polymorphism technique. ACR was defined as graft dysfunction, evidenced by elevated transaminase and/or bilirubin; their persistent initial elevation at least 3 times the upper normal limit in the absence of vascular or biliary complication or infection. The clinical suspicion of ACR was supported by protocoled mixed lymphocyte reaction (MLR) assay. Episodes of rejection were initially treated with either mini pulse (125–250 mg intravenous MP for 2–3 days or more) or with steroid pulse (500 mg intravenous MP for 3 days or more) according to clinical severity of ACR. Rejection was considered SRAR when liver function tests improved by <50% of the highest values after three steroid boluses. Sixteen out of the 102 (15.69 %) patients experienced ACR within 6 weeks after LDLT. The median time to ACR was 13.5 days. The median dose of total intravenous MP used was 937.5 mg (range: 500–3525 mg), and the median duration of treatment was 5 days (range: 2–8 days). Consistent with the incidence of SRAR in previous reports, five out of 16 (31.25 %) patients suffering from ACR were diagnosed as having SRAR. One of them was treated with OKT3, three with rATG, and one without OKT3/rATG. Six patients were diagnosed with ACR and received steroid bolus for rejection therapy. Liver biopsies of these patients after treatment showed that three patients responded well to steroid boluses and were thus clinically diagnosed with SSAR; indeed, the histological findings showed no evidence of rejection in all three patients. The other three patients, whose liver function tests improved by <50% of the highest values, were diagnosed with SRAR, and the histology revealed the remaining mild/moderate cellular rejection. We did not find any statistical association between the *FOXP3* SNPs genotype frequencies and the

incidence of ACR. However, significantly higher incidence of SRAR was observed in LT patients with the *FOXP3* rs3761548 A/C+A/A genotype than in those with C/C genotype (A/C+A/A vs. C/C; no rejection, SSAR, SRAR, 85.71%, 0%, 14.29% vs. 83.58%, 16.42%, 0%, respectively, $P = 0.0005$). The total dose of intravenous methylprednisolone used for ACR treatment was significantly higher in the rs3761548 A/C+A/A genotype than that in the C/C genotype (2037.5±887.5 gm vs 906.3±377.4 gm, $p=0.005$). DSA detection was carried out in the sera of 15 ACR patients. None of the patients had pre-formed DSAs. Two of the 10 (20%) SSAR patients with the rs3761548 C/C genotype developed de novo DSAs, whereas four of the five (80%) SRAR patients with the rs3761548 A/C+A/A genotype developed de novo DSAs. Although the difference between the two groups did not reach statistical significance ($P = 0.08$), these results suggest that ACR severity associated with de novo DSA formation is potentially influenced by the rs3761548 SNP. To examine the relationship between alloimmune responses and the *FOXP3* rs3761548 SNP, we monitored anti-donor alloreactivity at regular intervals by using an MLR assay employing an intracellular CFSE-labeling technique. Among patients who had never experienced ACR, the average anti-donor $CD4^+/CD8^+$ T cell Stimulation Index(SI) in patients with the rs3761548 A/C+A/A genotype was higher at all time points during the observation period than that in patients with the C/C genotype, although the difference did not reach statistical significance. When patients developed significant disorders in liver function as determined by laboratory tests after LT, MLR was performed for diagnosing ACR immediately after liver dysfunction had occurred. Anti-donor $CD4^+$ T cell SI determined by MLR assay at the time of ACR diagnosis was higher in patients with the rs3761548 A/C+A/A genotype than in patients with the C/C genotype, whereas the difference in anti-third party $CD4^+$ T cell SI between the genetically disparate two did not reach statistical significance. Thus, alloimmune responses in $CD4^+$ T cells during ACR after LT were more vigorous in *FOXP3* rs3761548 A/C+A/A individuals than in C/C individuals. We further investigated whether serum levels of immune-regulatory cytokines were influenced by the *FOXP3* genotype in LT patients. We found no significant association between serum levels of IL-2, IL-10, IFN-γ, IL-17A, or IL-35 and rs3761548 at any time point. Of note, among LT patients who had experienced ACR, serum IFN-γ levels at two weeks were markedly higher in patients with the rs3761548 A/C+A/A genotype than in patients with the C/C genotype. Infectious complications and overall survival were not related to *FOXP3* SNPs.

In conclusion, the *FOXP3* gene rs3761548 SNP in LT recipients was significantly associated with susceptibility to SRAR. This fact suggests that the immunosuppression regime and/or anti-ACR treatment regimen should be adjusted on an individual basis by identifying *FOXP3* SNPs, implying a need for personalized medicine in the field.