学位論文内容の要旨

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学 位 論 文 題 名

Augmented induction of a novel cancer/testis antigen in demethylated cancer cells
（脱メチル化処理がん細胞を用いた新規がん抗原の同定に関する研究）

Background and Objectives : Cancer immunotherapy including cancer vaccine has been considered as an attractive therapy for cancer patients because of the fewer side effects. CT (cancer/testis) antigens are widely expressed in various human cancers but their expressions in normal cells are tightly restricted in testis. Generally, it has been reported that cancer vaccine therapy using HLA class I-binding short peptide derived from CT antigens induced the antigen-specific CTLs and maintained the cancer patients at long stable disease. However, the vaccine therapy focused on only CTL activation appears to be suboptimal to conquer cancer. To overcome this problem, it is necessary to develop more efficient method to improve immunosuppression in cancer patients. It has been demonstrated that the introduction of Th1-dominant immunity in tumor-bearing host is essential for inducing fully activated CTLs and the subsequent memory T cell generation. 5-Aza-2′-deoxycytidine (DAC) is well known to induce gene expression of CT antigens by the demethylation of promoter CpG islands of the treated cancer cells. Therefore, we first used DAC-treated demethylated cancer cells to identify novel CT antigens in the present study and then tried identification of the helper peptide epitopes, applicable to development of more efficient cancer immunotherapy.

Methods : Human lung cancer cells, LC-OK, A549, and LC-MS cells were treated with or without DAC for 3 days. Gene expression levels of the A549 cells were evaluated by microarray analysis. The augmented gene in DAC-treated A549 cells were first selected as candidates, and the candidate gene expression levels in normal tissues were further evaluated by the multiple tissue cDNA panels including testis. C16orf73 gene, HP15 expressed in only testis, was selected as novel CT antigen in this study. HP15-specific $CD4^+$ T cells were induced from purified $CD4^+$ T cells by using HP15-overlapping peptides covering whole amino-acid sequence of HP15. HLA restriction of the HP15-specific $CD4^+$ T cells was further determined by the IFN-γ production in response to the peptide-loaded LCLs. HP15-specific T cells induced from PBMCs, were mixed with HLA-matched or mismatched lung cancer cell lines, which were pre-treated with or without IFN-γ and DAC in the presence or absence of anti-HLA class I and anti-HLA class II mAbs for 72 h. IFN-γ production by the established T cells was detected by ELISA. HP15 gene expression levels in various cancer tissues, primary cancer cells, and normal PBMCs were evaluated by quantitative- and RT-PCR.

Results: Gene expression levels of CT antigens, MAGE-A4, XAGE, and BAGE were augmented in human lung cancer cell lines, LC-OK, A549, or LC-MS cells after DAC-treatment. In addition, expression of a novel C16orf73 gene, HP15 was greatly enhanced in the demethylated lung cancer cells. The HP15 expression was observed in only testis but not other normal tissues, suggesting that HP15 was a novel CT antigen. HP15 gene expression was also detected in several cancer cell lines. Next, HP15-specific $CD4^+$ T cells were induced by using HP15-overlapping peptides. Then, helper

epitopes of HP15 CT antigen and the HLA restriction were identified by HP15-specific $CD4^+$ T cells and the overlapping peptides. As a result, almost all regions of HP15 CT antigen contained helper epitopes. Furthermore, it was confirmed that HP15-specific T cells established from PBMCs recognized HP15 antigen epitopes naturally processed and bond to HLA on human cancer cells in a HLA-class II dependent manner. In addition, the HP15-specific T cells did not respond to HLA-mismatched A549 cells, even if the cells were treated with DAC plus IFN-γ. HP15 gene was widely expressed in colon, lung, gallbladder, head and neck, and renal tissues from cancer patients. It was also confirmed that DAC-treatment enhanced HP15 gene expression level of primary cancer cells established from colon cancer patients but did not affect on that of normal PBMCs from healthy volunteers.

Discussion: In the present work, a novel CT antigen, HP15 was identified by DAC-treatment of human lung cancer cells. HP15 gene expression was observed in various cancer tissues of cancer patients as well as several cancer cell lines but not in normal tissues except testis. Therefore, the present strategy using demethylated cancer cells by treatment with DAC would be a useful tool to find novel CT antigens applicable to cancer vaccine therapy in human. It was also demonstrated that DAC-treatment caused the augmented induction of HP15 CT antigen in all cancer cells tested here as well as other CT antigens such as MAGE-A4 and XAGE, and BAGE in various cancer cells. These results suggested that DAC-treatment of cancer cells would increase their immunogenicity via augmented induction of CT antigens including our found HP15 CT antigen. To consider this possibility, helper epitopes of HP15 antigen were further identified, which can induce IFN-γ-producing $CD4^+$ T cells. In the present experiments, it was confirmed that HP15-specific $CD4^+$ T cells, induced by synthetic peptides, responded to the IFN-γ-treated and HLA class II-induced target cancer cells in a HLA class II- but not class I-dependent manner. These data suggest that such $CD4^+$ T cells would at least recognize the helper peptide epitopes naturally processed on HLA class II molecules of cancer cells. Recently, a clinical study for cancer patients revealed that DAC treatment induced NY-ESO-1 gene expression in the tumor tissues. In fact, HP15 gene expression was induced by DAC treatment in primary cancer cells from colorectal cancer patient. It was indicated that HP15 CT antigen was widely expressed in human cancer tissues in addition to human cancer cell lines. Taken together, the present results strongly indicate that DAC-treatment will become a novel strategy to induce immunogenic CT antigen, which facilitate the therapeutic efficacy of cancer vaccine treatment.

Conclusion: A novel CT antigen HP15 was identified in the present experiments and the gene expression was enhanced by DAC-treatment or spontaneously expressed in some cancer cells. In addition, it was confirmed that HP15 was expressed in various cancer tissues of patients. Therefore, demethylated cancer cells are useful tool to identify of novel CT antigens for cancer immunotherapy. Then, helper epitopes of HP15 antigen were determined by using HP15 overlapping peptides. In fact, HP15-specific $CD4^+$ T cells were successfully induced from PBMCs by HP15-overlapping peptides and the established T cells recognized HP15 and HLA-matched HLA-class II expressing cancer cells. These results indicate that HP15 is applicable to T cell based cancer immunotherapy. HP15 was widely expressed in colon, lung, gallbladder, head and neck, and renal tissues from cancer patients in addition to various cancer cell lines. Thus, these findings suggested that demethylation drugs such as DAC would be a useful tool not only for finding novel CT antigens but also for developing the combined therapy with cancer vaccine.