論文の要旨

論文題目 Prevention of Experimental Colitis-associated Colorectal Carcinogenesis by Vaccination with MUC1 DNA

(MUC1 DNA ワクチンによる大腸発癌の抑制)

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[Background and Aim]

Colorectal cancer is one of the most common malignant neoplasms. Pathogenesis of colorectal cancer is thought to be induced by genetic alterations accumulated under chronic inflammatory conditions in the large bowel. Effective preventive or therapeutic modalities against colorectal cancer are currently unavailable. Novel approaches such as cancer immunotherapy that target tumor associated-antigens offer an attractive alternative to the conventional cancer therapies, with the expectation of fewer side effects, and better prevention of metastasis and recurrence. MUC1 is a tumor associated-antigen over-expressed on various human colon adenocarcinoma and their precursor lesions. Immune response to MUC1 was detected in patient with pancreatic, breast, and ovarian tumors with the presence of antibodies and T cells specific for MUC1, and it was previously showed correlated to the better prognosis. Because of the highly increased expression and altered glycosylation in tumors, MUC1 is considered to be an effective target of cancer immunotherapy. Several studies aim to develop immunotherapy targeting on tumor associated antigens has been extensively conducted. We have previously reported that the growth of murine colon carcinoma SL4 cells-expressing MUC1 in the orthotropic site and lung metastasis of B16-F10 melanoma cells-expressing MUC1 were suppressed by vaccination with MUC1 DNA in C57BL/6 mice. However, those studies were conducted on transplantable models, which cannot represent the natural condition of tumor development. Therefore, it is very important to test the efficacy of MUC1 targeted immunotherapy in carcinogenesis model.

In this study, I employed MUC1 transgenic (MUC1.Tg) mice, which express endogenous MUC1 antigen, and have a similar level and pattern of MUC1 expression in cells and organs as seen in humans, to establish colitis-associated colorectal carcinogenesis model. As a MUC1 targeted therapy, I used MUC1 plasmid DNA (MUC1 DNA), which contained the full-length human MUC1 cDNA, combined with purified $CD11c^{+}$ bone-marrow derived dendritic cells (DCs) to increase its efficacy. The long-term goal of this study is to develop a MUC1 specific immunotherapy that targets tumor cells expressing MUC1, and thus preventing the development of colorectal tumor or inhibits its growth. The specific aims of this research are to establish colitis-associated colorectal carcinogenesis in MUC1.Tg mice, to evaluate the vaccination efficacy of MUC1 DNA combined with DCs (MUC1+DCs) in the prevention of colitis-associated colorectal cancer, and to elucidate its mechanism.

[Methods and Results]

1. Colitis-associated colorectal carcinogenesis model was established in MUC1.Tg mice.

1.1. Colitis-associated colorectal carcinogenesis in MUC1.Tg.

Azoxymethane-dextran sulfate sodium (AOM-DSS) colon carcinogenesis model is an experimental model to generate colitis-associated colon carcinogenesis in rodents. In this model, a single dose of AOM, a genotoxic carcinogen, will initiate tumor formation

in large bowel of rodents, followed by periodical administrations of DSS which induces inflammation that promotes carcinogenesis. After extensive optimization, I set up the condition for AOM-DSS colorectal carcinogenesis protocol to obtain spontaneous MUC1-expressing colorectal tumor growth in MUC1.Tg mice. Periodical administrations of 1% DSS after a single dose of 10μg/kg body weight AOM was effective to induce colitis-associated colorectal carcinogenesis in MUC1.Tg (100%-tumor incidence). The number of tumor per mouse was 11.25±3.57 and tumor size was 3.50±3.54 mm^2 respectively. Macroscopically, nodular and polypoid colonic tumors were observed in the distal colon of mouse and these tumors were histopathologically tubular adenoma, which can be characterized with various degrees of dysplasia, and squamous metaplasia with atypia.

1.2. Expression of MUC1 glycoforms in tumors induced by AOM-DSS.

To characterize MUC1 expression in the tumor developed in AOM-DSS colon carcinogenesis model, I performed immunohistochemical analysis by using various anti-MUC1 antibodies, which recognize MUC1 in different glycoforms. I found that 100% of adenomas (n=36) were positively recognized by chicken anti-MUC1 cytoplasmic tail polyclonal antibody (anti-CTP) which binds to MUC1 cytoplasmic tail, independent of glycoforms; 91.66% of adenomas showed strong binding to monoclonal antibody (mAb) MY.1E12 which recognize sialylated MUC1; 80% of adenomas were recognized by mAb HMFG1 which recognize glycosylated MUC1; and 91.18% showed binding to mAb HMFG2, which recognize poorly glycosylation MUC1. In the case of squamous metaplasia (n=10), all tumor tissues were recognized by all anti-MUC1 antibodies. Various MUC1 glycoforms are reported to be expressed in human colon adenomas and adenocarcinomas. The levels were correlated to the degree of malignancy and were useful in determining the prognosis of the disease. In the present study, I found that tumors developed in MUC1.Tg by AOM-DSS treatment also expressed various MUC1 glycoforms, similar to the MUC1 expression pattern in human. These results indicate that AOM-DSS colitis-associated colorectal carcinogenesis model in MUC1.Tg mice can be used as a suitable experimental model to evaluate the efficacy of immunotherapy targeting MUC1 expressing tumor.

2. Vaccination with MUC1+DCs suppressed colorectal carcinogenesis in MUC1.Tg mice.

2.1. Effect of MUC1 vaccination on tumor development.

To test the efficacy of MUC1 targeted immunotherapy, I next examined the preventive effect of MUC1 DNA vaccine in AOM-DSS colitis-associated colorectal carcinogenesis model. Mice were immunized three times at weekly intervals with 100 μg DNA plasmid mixed with or without $1x10^5$ DCs per mouse, followed by AOM-DSS administration. Vaccination with MUC1+DCs reduced tumor incidences in AOM-DSS treated MUC1.Tg mice to 37.5% reduction and decreased number of tumor per mouse in vaccinated MUC1.Tg mice compared to the treatment with control plasmid (pcDNA), MUC1 DNA plasmid (MUC1), DCs, or pcDNA combined with DCs (pcDNA+DCs). These results strongly indicate that combining MUC1 and DCs is critical to induce effective immunotherapy to inhibit tumor development in colitis-associated colorectal carcinogenesis model.

2.2. Effect of MUC1 vaccination in different types of tumors.

To further determine whether MUC1 targeted immunotherapy was effective against particular type of tumor, I characterized the tumor by histological examination into adenoma with low-grade dysplasia, adenoma with high-grade dysplasia and squamous metaplasia with atypia. The development of all types of tumors was suppressed in MUC1+DCs-vaccinated mice. Adenoma with low-grade dysplasia observed in MUC1+DCs-vaccinated mice, showed reduced levels of MUC1. However adenoma with

high-grade dysplasia and squamous metaplasia with atypia that were rarely found in MUC1+DCs-vaccinated mice maintained the levels of MUC1 similar to control groups. These results show that vaccination with MUC1+DCs are effective regardless of the malignancy of tumor in AOM-DSS colon carcinogenesis model, and MUC1 expression were maintained even in tumors developed in MUC1+DCs-vaccinated mice.

3. Vaccination with MUC1+DCs induced MUC1-specific immune response.

3.1.Induction of antigen-specific antibody production after MUC1 vaccination.

To elucidate the mechanism of the MUC1+DCs vaccine effects against colon cancer development, I evaluated anti-MUC1 immune responses elicited by vaccination with MUC1+DCs. For the measurement of humoral immune responses, I determined MUC1-specific antibody production in the sera after MUC1 vaccination. There was a significant increase in MUC1-specific total Ig (IgG, A, and M) production in the sera by ELISA in MUC1+DCs-vaccinated group, compared to before vaccination, and vaccination with pcDNA, MUC1, and DCs alone. MUC1+DCs-vaccinated micesignificantlyhave higher MUC1-specific antibody production compared to pcDNA+DCs-vaccinated mice.

3.2. Induction of antigen-specific cellular response after MUC1 vaccination.

I next examined whether vaccination with MUC1+DCs induced cellular immune response against MUC1. MUC1-specific IFN-γ secretion by splenocytes from MUC1+DCs-vaccinated mice was quantified by ELISPOT assays. MUC1+DCs-vaccinated mice showed significantly higher frequency of MUC1-specific IFN-γ producing cells compared to control group ($p=0.0067$). These results strongly suggest that MUC1-specific IFN-γ producing T cells are elicited in MUC1.Tg mice vaccination with MUC1+DCs. Collectively, these results indicate that MUC1-specific cellular immune responses are responsible for the inhibition of tumor development by vaccination with MUC1+DCs in AOM-DSS colitis-associated colorectal carcinogenesis.

[Conclusions]

In the present study, I applied carcinogen-initiated and inflammation-driven colon carcinogenesis to MUC1.Tg, and demonstrated for the first time that the tumors expressed MUC1. I clearly demonstrated that significant reduction of carcinogenesis was observed when DCs were combined with MUC1 DNA vaccine and administrated to these mice prior to tumor development. The suppression of tumor induction was likely to be due to MUC1-specific immune responses. These results implicate the potential of this novel immunotherapy approach as a novel therapy to prevent colorectal carcinoma growth in clinic.

This study is the first to report the efficacy MUC1 DNA combined with DCs in the prevention of colitis-associated colorectal carcinogenesis in MUC1.Tg mice. This vaccine strategy also highlight the beneficial contribution of DCs to enhanched antitumor immunity of MUC1 DNA vaccine.

This study provides supporting preclinical evidence on the efficacy of MUC1 DNA and DCs vaccination to prevent colitis-association colorectal carcinogenesis. These findings support additional investigation on combination of MUC1 DNA and DCs for the prevention of colorectal cancer in a human. The results of this study also support our previous reports on the suppression of the MUC1-transfected colon tumor growth and MUC1-transfected lung metastasis by using MUC1 DNA vaccine in wild type mice. Future study should be conducted to elucidate the detailed mechanism on how this vaccination break the tolerance, and how to increase the potency of this vaccine.