Generation of tumor vaccines both in vitro and in vivo for cancer immunotherapy
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Cancer is one of the most common causes of death in the world. Escaping of malignant cells from host immune system “immune surveillance” is considered a key element for tumor development and metastasis. The mechanism(s) by which immune tolerance to cancer cells developed in hosts still remains a big challenge for the scientists and oncologists and needs urgently to be further elucidated. It will provide a foundation for cure of cancers in the future if these basic questions can be answered in detail. We successfully developed a novel approach to correct these defects. The concept of this approach is to fusion of tumor cells with professional antigen presenting cells. The tumor cells generated by this fusion approach became more immunogenic and could effectively stimulate host immune system to develop an antitumor specific immunity, which were both preventable and curable even in these hosts who had developed an immune tolerance to tumors. These results suggested that this novel approach was able to correct the existed impairment of antigen processing function in tumor cells and made them immunogenic by processing and presenting the hidden tumor antigen to host immune system. The expression of MHC and costimulatory molecules could be significantly enhanced by in vitro treatment of tumor cells with combination of cytokines and bispecific monoclonal antibodies with a binding site to tumor antigen expressed on tumor cells and another to costimulatory molecules on immune cells, which could provide strong signals for activation of T cells. Modification of tumor cells with this “two step process” can generated tumor vaccines for cancer immunotherapy. To further investigate approaches to gene rating tumor vaccines in vivo, we developed several novel bispecific antibodies, which consisting of two normal binding sites for targeting antigens and a functioning portion of Flt3L. The constructed bispecific antibodies efficiently target hepatoma, breast and lymphoma cancer cells in vitro and in vivo, leading to accumulation of DCs,NK cells and lymphocytes in local tumor tissues. Administration of these bispecific monoclonal antibodies can protect bone marrow injury caused by chemotherapeutic drugs and stimulates proliferation and maturation of lymphocytes, APCs and NKs. Systemic administration of these bispecific antibodies significantly inhibited tumor growth and cured established tumors. These bispecific therapeutic proteins are effective in elicitation of long-lasting antitumor immunity. The tumor specific immunity can be adoptively transferred into naive animals successfully by transfusion of CD3+CD8+ T cells from the treated mice. The results suggest that effective tumor vaccines can be successfully generated by modification of tumor cells or APCs both in vitro and in vivo and these novel approaches may provide novel strategies for treatment of cancers.

IGF-I Inhibition and CD9 Modulation in Melanoma Immunotherapy
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IGF-I (Insulin like growth factor I), a pleiotrophic factor intervening in many biological processes is expressed in most tumors. By using anti-sense vector or specific antibody, we have modified a melanoma cell line, the B16-F0 from C57BL/6 mice (hence termed B16-F0.MOD), in blocking IGF-I expression. The modified tumor cells were characterized not only by the absence of IGF-I, but also by a low expression of CD9, a member of the tetraspanin family. Furthermore, they were resistant to MHC-I stimulatory effects of interferon gamma and had a lower in vivo proliferation rate as compared to wild type counterparts (B16-F0.WT). Syngeneic hosts vaccinated with the modified cells developed humoral as well as cellular immune effectors able to control in vitro and in vivo tumor proliferation.

Immuono target modulation for breast cancer stem cells
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Background: Recent studies have confirmed that breast cancers are originated from a cell subpopulation with characteristics of self-renewal and differentiation named breast cancer stem cells. It is generally acknowledged that CD44+/CD24− is the marker for breast cancer stem cells. The abnormal activities of some signal transduction pathways, such as Notch, Wnt and Hedgehog make up of the network regulating the self-renewal of breast cancer stem cells. The management of breast cancers are transforming from killing cancer cells to eliminating or differentiating breast cancer stem cells. The treatment for targeting breast cancer stem cells involve targeting signal pathways, differentiating therapy, and immunotherapy and so on.

Methods: CD44 and CD24 expression were determined by flow cytometry. CD44+/CD24− breast cancer stem cells were isolated from MCF-7 and MDA-MB-231 breast cancer cell lines, which were performed RT-PCR to clarify the expression of WNT1/β-catenin, Notch-1/HE, Hedgehog (Shh, Gli1, PTCH) and ABCG2. Meantime, such subpopulation was also determined from the apheresis from patients who undertaken to high dose chemotherapy to compare the amount of such population with the clinical treatment response as well as progression free survival time. The cancer cell differentiation reagent of demethylation, uroacitide was incubated with CD44+/CD24− breast cancer stem cells to testify whether it has activities against breast cancer stem cells.

Results: The percentage of CD44+/CD24− in MCF-7 and MDA-MB-231 cell lines is shown to be 0.5-1.0% and 15-30% respectively. CD44+/CD24− breast cancer stem cells have higher expression in β-catenin, Notch-1, Hes-1, ABCG2 than other cells in both cell lines.