

Lupu Catalin Mario

Dr. med.

### **Prevention of Liver Metastasis in Colon Cancer Bearing Mice by Adenovirus B7-H3 Gene Therapy**

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Staats Examen am 1999 an der Universität Medizin Victor Babes Timisoara Rumänien

Promotionsfach: Innere Medizin

Doctorvater: Priv.-Doz. Dr.med Jens Encke

Although for colon cancer primary tumors can usually be surgically removed, the outcome of current therapies is often unsuccessful in preventing metastases. In the search for novel therapeutic approaches to treat patients with locally advanced colorectal carcinoma, gene therapy holds promise. Efficiency of gene therapy as a treatment approach for colon cancer depends on type of active genes inserted, how the gene was delivered and how is express the therapeutic genes at the site of cancer. Many studies have demonstrated that the immune system can be polarized against malignant cells by inducing costimulatory molecules into tumor cells.

The B7-H3 molecule was very recently discovered and belongs to the family of B7 costimulatory molecules. Her role in induction of specific T-cell proliferation, cytotoxic T-cell activation and cytokine production is controversial.

The current notion that tumor cells can “escape” immune recognition is based on previous studies which proved that lack of costimulatory molecules on tumor cells induce T-cell anergy. Moreover the tumor microenvironment plays a critical factor in the balance between tumor growth and immune control.

Therefore in the present study we have successfully established a simple, reproducible and consistent orthotopic animal model for colon cancer using the Colon-26 murine adenocarcinoma cell line.

We used our model to study the antitumoral effect of B7-H3 on colon cancer and we could prove that the presence of the costimulatory molecule plays an important role for induction of specific immune responses against tumor cells. After orthotopically implantation of non immunogenic C26 tumor cells and C26 cells which contain the transfected B7-H3 gene, tumors grew progressively in both groups of immuno competent syngeneic mice, but presence of B7-H3 decreased tumorigenicity and slowed tumor growth. We have demonstrated enhanced levels of IL-2 in C26-B7-H3 tumor bearing mice and showed that B7-H3 plays an important role in the initiation of CTL-mediated anti-tumor immunity *in vivo*.

Moreover, using our orthotopic model we have investigated whether direct intra-tumoral injection of a recombinant adenovirus expressing the B7-H3 costimulatory molecule, could result in anti-tumor immune responses capable of affecting tumor progression and metastasis occurrence. We have analyzed serum levels of IL-12 cytokine also known as NK-cell stimulatory factor or cytotoxic lymphocyte maturation factor in C26 tumor bearing mice treated with either Ad-B7H3-GFP or Ad-GFP. We found that mice treated with Ad-B7H3-GFP showed a marked increase in serum IL-12 levels that can induce proliferation and cytotoxic activity of T-cells and NK cells.

Our results shown that treatment efficiency was different reported to tumor volume but after treatment tumor volume significantly decreases in group treated with Ad-B7-H3-GFP compared with Ad-GFP group were tumor growth until mice become moribund. Moreover, long-term secondary metastasis occurrence was found to be reduced in mice treated with Ad-B7-H3-GFP as compared with those treated with Ad-GFP.

Using two series of experiments, C26-B7-H3 transfectants orthotopic injected and C26 established tumor injected with Ad-B7-H3 we could prove that the presence of the costimulatory molecule plays an important role for induction of specific immune responses against tumor cells. We could show that the orthotopically introduced costimulatory molecule can activate naive T-cells to become tumor specific effectors T-cells secreting IFN- $\gamma$ .

In conclusion although the utility of adenovirus vectors for use in clinics has been seriously challenged in the last two years, they represent an attractive strategy for therapy of patients with advanced colon cancer who acquired resistance to current therapeutic protocols. Overall, our results support the feasibility of this approach and justify further investigation of this strategy for colon cancer therapy. Further studies are needed to reconcile the results of studies showing inhibitory and stimulatory functions for B7-H3. Such results could be explained by the existence of two receptors for B7-H3 with opposing functions, similar to CD28 and CTLA-4.