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Tri-ART: TriMix and Antigen mRNA-based anti-cancer Therapy

Introduction

Cancer is a disease for which it is challenging to define effective treatment regims and unfortunatelly still remains one of the major causes of death in our industrialized world. Today, first line treatment is surgery, radio- and chemotherapy. Despite major improvements in these conventional treatment strategies, it is imperative to develop novel treatment methods that are tumor cell specific and provide long-term protection to avoid recurrence of tumor cells.

It has been suggested that cancer immunotherapy is a powerfull strategy that oncologists can offer to patients. The main premise of cancer immunotherapy is to harness the patients own immune system to specifically recognize and kill tumor cells. This is based on the knowledge that our immune system can discriminate healthy cells from cancer cells as the latter express tumor-associated antigens (TAA). In this regard, dendritic cells (DC) and T cells play an important role. DCs, professional antigen presenting cells, need to be loaded with these TAA in order to induce cancer specific cytotoxic T lymphocytes (CTL). Moreover DCs need to be activated in order to present their antigenic cargo in the presence of co-stimulatory molecules.

Dendritic cell based immunotherapy: from ex vivo towards in vivo application

The host laboratory 'Laboratory of Molecular and Cellular Therapy' is mainly involved in the field of cancer immunotherapy. We have evaluated the exploitation of DCs for immunotherapy. The research performed in our laboratory has led towards a standard protocol for the generation of an efficient DC-based vaccine. We developped a protocol by which DCs are loaded with antigen and maturated in a one-step procedure by means of using mRNA encoding TAA, the co-stimulatory molecule CD70 and the activation signals CD40 ligand (CD40L) and a constitutively active form of Toll like receptor 4 (caTLR4). The mix of DC modulating mRNA molecules is called TriMix. Moreover, we showed that vaccination of melanoma patients with TriMix-DCs induced tumor antigen specific CTLs and resulted in clinical responses in a number of patients.

During my PhD, I am actively persuing the use of mRNA for the in vivo loading with antigen and activation of DCs. With this research we want to circumvent the use of the expensive and time-consuming patient-specific DC-vaccine. The aim of my research is to evaluate the use of TriMix and TAA mRNA as an off-the-shelf vaccine. In this regard, we showed that intranodal injection with mRNA results in the uptake by DCs. Moreover, we showed DC activation and the induction of antigen-specific CTL after IN injection of TriMix and antigen mRNA. This resulted in a reduced tumor growth and a prolonged survival of tumor-bearing mice. We

compared intranodal immunization with mRNA with intradermal injection, showing superior results for intranodal vaccination. Finally, mRNA vaccination is as efficient in CTL induction and therapy response as vaccination with mRNA electroporated DCs. These findings suggest that intranodal administration of TAA mRNA together with TriMix is a promising vaccination strategy.

In the future, we want to further explore the role of mRNA as an off-the-shelf immunotherapeutic. It is well accepted that the tumor micro-environment is dominated by tumor induced interactions which keep DCs immature or trap them within the tumor resulting in impaired effective presentation of ingested antigens to T cells and subsequently resulting in an impaired immune response. It is our aim, by intratumoral delivery of TriMix mRNA, to rescue DCs from these suppressive activities. In this regard, we first want to evaluate the effect of intratumoral delivery of TriMix on the tumor micro-environment and on the tumor-draining lymph nodes. During these experiments, the cytokines provided by ImmunoTools could be of great help. Since DCs play a major role in the induction of an effective immune response, we carefully want to analyse the effect of TriMix on DCs. Several cytokines provided by ImmunoTools play an important role in this process and will be analysed. Next, we will analyze the induction of systemic immune responses. In this process CTLs play a major role, hence we will go into more detail and screen for the upregulation and secretion of several markers of which most of them are included in the panel of ImmunoTools.

mRNA: from chemical blueprint for protein production towards an anti-cancer vaccine.
Today, mRNA is recognized as an active pharmaceutical ingredient for the treatment of cancer. This is already shown by the publication of several clinical trials using mRNA for cancer treatment. Our research regarding the in vivo use of mRNA forms the basis for a clinical trial by which patients with hepatocellular carcinoma will be vaccinated with mRNA.

The growing interest in mRNA is explained by its versatility and the many advantages it offers. The use of cancer vaccines based on mRNA may offer a solution as clinical grade material, which could be produced reliably and rapidly in a scalable process adressing substantional needs in cancer vaccinology. We believe that our findings regarding the direct application of TriMix and/or TAA mRNA will open attractive perspectives for immunization. Moreover, we believe that the gift offered by ImmunoTools will be of great help during our future research.