Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Microscopic study of RNA-lipoplex-mediated cell targeting using 2D and 3D models

Autor: Maximilian E. A. Schäfer
Institut / Klinik: Institut für Molekular- und Zellbiologie der Hochschule Mannheim
Doktorvater: Prof. Dr. M. Hafner

Advanced mRNA therapeutics are not only emerging as vaccines, but are also being investigated for cancer therapy. The purpose of RNA lipoplexes (lipid nanoparticle-based RNA therapeutics) in tumor immunotherapy is to encode tumor-associated antigens and deliver this information to antigen-presenting cells (APCs). Besides the systemic application of RNA-LPX with the intention of targeting APCs in organs of the lymphatic system such as the spleen or lymph nodes, local applications in the direct tumor environment are also being studied.
The presented work describes the cell targeting of an RNA-lipoplex based therapeutic in different modalities. The focus is placed on *in vitro* and *in vivo* studies that reflect direct as well as systemic application. For data acquisition, microscopic analyses were used with a special focus on 3D microscopy. The studies revealed varying colocalizations with different cell types in the different study models.
In the described *in vitro* melanoma model, different cell type specific RNA reporter expression could be observed in classical 2D cell culture as well as in 3D mono and cocultures. The 2D cell cultures indicated an increased expression in the coculture compared to monocultures. In 3D monocultures, increased eGFP (RNA reporter) signal was detected in the SK-MEL 28 cells. In the 3D coculture system, a distribution within the spheroid could be depicted, where in addition to the CK 14 (basal keratinocytes) positive cells localised in the rim area, also eGFP was expressed in the spheroid nucleus.
Due to its central role in the secondary lymphatic system, the spleen was analysed as a target organ for systemic application, both in conventional 2D sections and in 3D whole mounts. Based on the results of the 2D sections, we found that both CD11c positive and CD169 positive cells show strong colocalizations with the RNA reporter signal. However, among APCs an increased colocalizations with CD169 positive cells could be detected.
For a comparison between classical 2D analyses and state-of-the-art 3D analyses, different techniques were tested and a method was adapted to the special requirements of the target organ (spleen) and the detection of eGFP (RNA reporter). These studies have provided important insights into the strength of the tested tissue clearing techniques. Furthermore, colocalization between eGFP and CD169-positive cells in 3D provided a first insight into quantitative analyses like those from 2D tissue sections. The described method can be implemented using standard tools and thereby opens the way for future 3D studies of lipid nanoparticle-based RNA therapeutics.