Abstract

CD47 is a so-called „don´t eat me“ signal, which protects cells from phagocytosis. Its high expresion on tumor cells brings new perspective to the tumor therapy. Monoclonal antibodies, which are these days undergoing clinical trials, prevent CD47 binding to the SIRPA inhibitory receptor on macrophages, and so they enhance their phagocytic functional capacity. In this way they enable phagocytic removal of tumor cells. Overall expression, structural conformation and stoichiometry of CD47 on a particular cell predestine whether it will be phagocytised.

The aim of the thesis is to develop and test methods to characterise expression parameters of CD47 via flow cytometry (FCM), quantitative PCR (qPCR) and microscopy. To achieve this goal I performed competition tests of commercially available antibodies in order to characterise their binding epitopes on cell lines. After performing tSNE analysis of primary BCa patient samples I correlated CD47 expression with other cell surface markers. I focused on CD47 expression in various differentiation stages of the tumor. To better understand the relationship between CD47 expression and differentiation status of cells I performed qPCR analysis of particular transcription factors. Using cell lines I examined method for phagocytosis quantification, which will be used to measure phagocytosis rate in patient samples in the future.

Key words: CD47, surface expression, 3D microscopy, transcription program, phagocytosis, macrophage, carcinoma, cancer stem cell, tumor immunotherapy