Twan Lammers
Dr. sc. hum.

Evaluation of the role of PP2Cα in cell growth, in cellular stress signaling and in tumorigenesis

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Promotionsfach: DKFZ / Clinical Cooperation Unit Radiation Oncology
Doktorvater: Prof. Dr. med. Dr. rer. nat. Jürgen Debus

The purpose of the present thesis was to evaluate the role of the prototypic type 2C phosphatase PP2Cα in cell growth, in cellular stress signaling, in radio- and chemosensitivity, and in tumorigenicity. Hereto, the proliferation rate, the clonogenic survival, the membrane integrity and the cell cycle distribution of wild type and PP2Cα siRNA-expressing MCF7 were investigated, both under basal conditions and upon treatment with different doses of radio- and chemotherapy. The effect of the knockdown of PP2Cα on cellular stress signaling was evaluated by means of western blot analysis. A proteomic approach, comprising 2-D protein separation, computer-assisted image analysis and MALDI-TOF mass spectrometry, was used to identify new PP2Cα target proteins. The role of PP2Cα in tumorigenesis was assessed by comparing the growth of tumors established from wild type MCF7 cells to that of tumors established from PP2Cα knockdown MCF7 cells. No macroscopic differences in proliferation, in clonogenicity and in membrane integrity were observed between wild type and PP2Cα siRNA-expressing MCF7 cells, neither under basal conditions, nor upon treatment with radio- and chemotherapy. Under basal conditions, the knockdown of PP2Cα decreased the number of cells in the G0/G1 phase of the cell cycle, and it increased their percentages in G2/M. In line with this finding, also upon treatment with radio- and chemotherapy, the percentages in G0/G1 and in G2/M were affected. The expression levels of MDM2, of phosphorylated Akt and of VEGF were found to be increased upon the knockdown of PP2Cα. Upon radiotherapy-induced stress, p53 signaling was found to be distorted in PP2Cα siRNA-expressing MCF7 cells. Proteomic analyses identified GRP-78, ER-60 and AK5 as previously unrecognized target proteins of PP2Cα. Upon the combined implementation of estrogen-containing hormone pellets and Matrigel, the tumorigenicity, the proliferation rate of the tumors, and the degree of pericyte coverage of the tumor blood vessels were found to be higher for PP2Cα knockdown tumors. These results demonstrate that the prototypic type 2C phosphatase PP2Cα does not play a role in regulating cell growth in vitro, at least not in MCF7 cells. In addition, it is not involved in determining the radio- and chemosensitivity of these cells. The siRNA-mediated knockdown of PP2Cα reduces the number of cells in the G0/G1 phase of the cell cycle, it attenuates the induction of the G1 block, it increases the number of cells in G2/M, and it enhances the induction of the G2 block. MDM2, Akt, VEGF, GRP-78, ER-60 and AK5 are identified as novel target proteins of PP2Cα. The tumorigenic potential of PP2Cα knockdown MCF7 cells is higher than that of wild type MCF7 cells, and the vasculature in these tumors is better differentiated. Based on these findings, we conclude that PP2Cα plays a role in the regulation of the cell cycle, in the induction of cell cycle checkpoints, in tumorigenesis and in angiogenesis.