P71 Primary cell cultures of different renal tissue species as a representative in vitro model for translational research?

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Introduction & Objectives: Renal cell carcinoma (RCC) is a challenging cancer with limited therapeutic options beside surgery. For translational research, we generated primary cell cultures of benign and malignant renal tissue entities. The expression of hypoxia-activated genes, genes encoding for growth receptors, mitophagy and immune system evasion were analyzed to assess their similarity to the tissue they derived from, their adaptation to cell culture conditions and to a new microenvironment.

Materials & Methods: 15 cases of clear cell renal cell carcinoma (ccRCC), 5 cases of papillary RCC (pRCC), 1 case of oncocytoma and the corresponding benign tissues were included. After tissue digestion, filtration and cultivation, genomic DNA and mRNA were extracted. The cells were validated using IHC staining for specific tumor markers of ccRCC and pRCC, respectively. ccRCC samples were subjected to mutational analysis of the 3 VHL exons via Sanger sequencing. RT-qPCR was performed for VHL, HIF-1α and CA9 as hypoxia associated genes; PINK1, PARK2 and PACRG for mitophagy; EGFR-1, NRP-2, VEGF-A and VEGF-C for growth factors and receptors; PD-L1 as an immune checkpoint molecule. We compared the expression of the primary cell cultures with the expression of the tissue of origin.

Results: 12/15 ccRCC samples, all 5 pRCC and all cases of benign tissue as well as the oncocytoma could be cultivated. ccRCC cultures expressed tumor markers of ccRCC (CA9 and/or CD10), cultures of pRCC expressed specific pRCC markers (CK7, AMACR). Cell cultures of benign tissue were positive for PAX8 and vimentin as renal-epithelial markers. 4/7 ccRCC primary cell cultures tested for VHL gene abnormalities had mutations which matched the tissue of origin. All cell cultures of ccRCC, pRCC and non-neoplastic tissue had increased levels of hypoxia-associated genes (VHL, HIF1A, CA9) and PD-L1, involved in immune evasion under cell culture conditions. Expression levels of mitophagy-related genes PARK2 and PACRG were reduced, while PINK1 showed variable expression. RCC cell cultures had similar levels of growth factor and receptor expression compared to their tissues of origin, elevated expression was observed in benign tissue cell cultures.

Conclusions: The successfully generated primary cell cultures of renal tissue entities displayed adaption mechanisms like pseudo-hypoxic reactions, a change of mitochondrial metabolism and enhanced immune evasion. Especially the adaption to hypoxia and immune evasion are known resistance factors of RCC. Their investigation might provide new approaches for targeted therapies which are urgently needed to face these challenging renal cancer entities.