SUMMARY OF THE DISSERTATION: MOLECULAR BIOLOGICAL CHARACTERIZATION OF AGGRESSIVE PARAGANGLIOMA

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Paragangliomas (PGLs) are tumors of chromaffin cell origin, including adrenal-derived pheochromocytomas (PHEOs). Diagnosis of PHEO/PGL remains a challenge, because signs and symptoms are not characteristic. If untreated, PGL can have a devastating outcome due to catecholamine excess. Even after proper diagnosis, the risk of metastatic disease remains. In up to 10 % of patients, metastases are present at diagnosis and metastases occur in 50-97 % of patients with underlying succinate dehydrogenase B (*SDHB*) mutations. Once metastatses are present, treatment options are limited.

The work presented here aims at better characterization of metastatic PGL on the molecular biological level. First, the value of urinary peptide patterns was evaluated for classification of selected patient groups to potentially improve diagnostic means. The urine of healthy control persons (Ctr), patients with *SDHB*-derived metastatic (*SDHB*-met) and non-metastatic (*SDHB*–ben) PHEO/PGL and healthy *SDHB* mutation carriers (*SDHB*–car) was analyzed by capillary electrophoreses coupled electrospray ionization mass spectrometry. Based on the differences in urinary peptide patterns, possible predictive markers for *SDHB* mutation and differentiation between *SDHB*-car and *SDHB*-ben or *SDHB*-met were identified.

Classification of *SDHB*-car was successful based on 4 peptides (error rate 1.8 %). *SDHB*-met were correctly classified with an error rate of 3.3 % based on only 1 peptide. The top ranking peptide was identified as a collagen type III fragment. Separation of *SDHB*-car&ben using 3 peptides yielded an error rate of 10.5 %. However, by combining the 3 top-ranked peptides with presence/absence of elevated plasma catecholamine levels, 100 % correct classification was reached. Thus, urinary peptides hold the potential to detect *SDHB* mutation and to distinguish mere mutation carriers from patients with *SDHB*-derived metastatic PHEO/PGL. In addition, our data suggests that combining catecholamine testing with evaluation of 3 urinary peptides may be beneficial in diagnosing non-secreting *SDHB*-derived PGL.

Second, distinct protein expression patterns in aggressive PGL were evaluated. The focus was on further exploring dysfunction in energy metabolism related pathways. PHEO/PGL derived due to *SDH*x and von Hippel-Lindau (*VHL*) gene mutations have previously been associated with strengthened glycolysis and decreased oxidative phosphorylation (OXPHOS). Nevertheless, tumor aggressiveness is distinct: metastases rarely occur in *VHL*-derived PHEOs/PGLs, while *SDHB*-derived PHEOs/PGLs often metastasize. Differential 2D-gel analysis of mouse PHEO cells (MPC), and their more aggressive filial mouse tumor tissue (MTT) cells, revealed changed expression of several energy metabolism proteins, including ATP synthase. These were then assessed in human PHEOs/PGLs. In support of a glycolytic phenotype, lactate dehydrogenase was elevated with an increased A to B subunit ratio in MTT compared to MPC; this was also the case for *SDHB* relative to human normal adrenal medulla. A to B ratio was similarly elevated in *SDHB*- and *VHL*-derived tumors, however due to an A subunit increase in *SDHB* and B subunit decrease in *VHL*. Glucose starvation reduced proliferation of MTT, but not MPC, confirming increased dependence on glycolysis in the more aggressive cells. However, respirometry revealed no difference in oxygen consumption at complex I and II, while ROS production at complex I was increased in MTT cells. Evaluation of OXPHOS activity in human tissue revealed reduced complex I, II and II+III activity in *SDHB* when normalized to mitochondrial content. However, no difference in ROS levels was observed. These results show that the aggressive MTT and *SDHB*-derived PHEOs/PGLs share certain characteristics when compared to their less aggressive counterparts. Pathogenic mechanisms of aggressive human PHEOs/PGLs related to the Warburg effect, which may lead to identification of new diagnostic and prognostic markers and potential therapeutic targets, are described.

Third, a possible cell surface location of ATP synthase - which has been reported for other cancers - and its potential as therapeutic target was evaluated. Confocal microscopy revealed that ATP synthase was present on the cell surface of MPC and MTT cells as well as primary cells of an *SDHB*-derived PGL, while virtually absent on bovine primary chromaffin cells. Cell surface location of ATP synthase β was verified in tissue of an *SDHB*-derived PGL by immunoelectron microscopy. Treatment of MPC and MTT cells with resveratrol as well as ATP synthase β antibody lead to severe proliferation inhibition. The presented data suggest that PHEO/PGL carry a functional ATP synthase complex on their surface that promotes cell survival or proliferation. Thus, cell surface ATP synthase may present a novel therapeutic target in treating metastatic disease and inoperable PHEO/PGL.