Interplay of hypoxia signaling and kynurenine pathway in PPGLs

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Pseudohypoxic cluster 1 pheochromocytomas and paragangliomas (PPGLs) display more aggressive disease progression than cluster 2 PPGLs, which are characterized by kinase signaling activation. Enhanced stabilization of hypoxia inducible factor 2α (HIF2α) in pseudohypoxic PPGLs leads to expression of HIF target genes and changes in the tumor microenvironment. However, the exact mechanism(s) underlying the increased metastatic potential of cluster 1 PPGLs remain unclear. We hypothesized that hypoxia-associated metabolic reprograming contributes to the aggressive phenotype of pseudohypoxic PPGLs. To investigate this, we focused on the kynurenine pathway (KP), a major branch of tryptophan (TRP) metabolism. Kynurenine (KYN) and several of its downstream metabolites can act as ligands for the aryl hydrocarbon receptor (AHR) that similar to HIF1α and 2α, can form a complex with aryl hydrocarbon receptor nuclear translocator (ARNT) and activate either AHR/ARNT or HIF/ARNT signaling. Thus, the aim of this study was to characterize the interplay between HIF and KYN pathways in PPGLs. Analysis of TRP and of KP-related metabolites in PPGL tissues revealed a pronounced shift towards KYN in cluster 1 versus cluster 2 PPGLs. Cluster 1 PPGLs exhibited increased expression of the rate-limiting enzymes in the KP, indoleamine 2,3-dioxygenase (*IDO1*), and tryptophan 2,3-dioxygenase (*TDO2*), in comparison to cluster 2 PPGLs. To validate the hypothesized crosstalk with the HIF pathway, human-derived and mouse pheochromocytoma cells (hPheo1 and MPC) were cultivated under normoxic or hypoxic (≤1% O_2) conditions. In hPheo1 cells expressing HIF1α and HIF2α, extrinsic hypoxia shifted the KP towards TRP accumulation and KYN reduction via reduced *TDO2* expression. MPC cells expressing *Hif2a*, exhibiting a more aggressive phenotype, showed no differences in TRP or KP-related metabolite levels compared to the less aggressive control cells lacking *Hif2α* expression under both normoxic and hypoxic conditions. Furthermore, KYN levels were significantly decreased in adrenal tissue of TH:cre-PHD2/PHD3$^{f f/f f}$ mice, where both Hif1α and Hif2α are stabilized, compared to the wild type mice. No differences in KYN metabolism were observed in adrenal tissue of TH:cre-PHD2/HIF1$^{f f/f f}$ mice characterized by Hif2α stabilization. Our findings suggest that Hif1α, rather than Hif2α, is involved in KP regulation, indicating that differences observed in PPGL tumor tissues may be caused by more complex processes, possibly related to changes in the tumor microenvironment.