Developmental role of PHD2 in the pathogenesis of pseudohypoxic pheochromocytoma

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BACKGROUND

Hypoxia inducible factors (HIFs) play an important role in physiological adaptations to low oxygen, however, their excessive activation can result in different pathologies including cancer. One example in which the latter is the case, are so called 'pseudohypoxic' tumours of the autonomic nervous system that arise in the adrenal medulla (pheochromocytomas, PCCs) and extra-adrenal sites (paragangliomas, PGLs). PCC/PGLs have the highest degree of heritability in human neoplasms, with up to 40% of these tumours being associated with a germline mutation (Buffet *et al*., 2020). Molecular analysis of these tumours has revealed a number of subtypes with distinct patterns of gene expression within a tumour being associated with different groups of tumour-associated mutations (Crona *et al*., 2017, Fishbein and Wilkerson, 2018). The pseudohypoxic subtype of PCC/PGLs is associated with mutations affecting transcriptional pathways induced by hypoxia (e.g. gain-of-function mutations of *HIF-2α* itself or loss-of-function of its negative regulators *VHL* or *PHD2,* Zhuang *et al*., 2012, Dahia *et al*., 2005, Ladroue *et al*., 2008). This has led to the question whether activation of HIF is driving oncogenesis, at least in these tumours.

OBJECTIVE

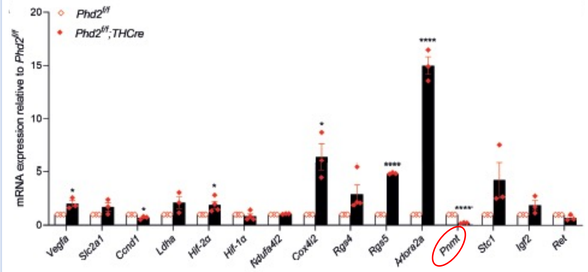
Gain insight into the pathogenesis of pseudohypoxic pheochromocytomas.

MATERIALS & METHODS

We have previously described the *Phd2$^{f/f}$;THCre* mouse model with constitutive but tissue-restricted inactivation of the *Phd2* gene in TH$^+$ cells, including those of the adrenal medulla (AM). Mice were studied at an age of ~3 months. We have analysed morphological and gene expression changes in AMs of *Phd2$^{f/f}$;THCre* using quantitative (qPCR) and qualitative (immunohistochemistry and *in situ* hybridisation) techniques. We further applied a competitive enzyme immunoassay to test AM function by measuring plasma catecholamine levels. Histological findings of morphological abnormalities and altered spatial gene expression in *Phd2$^{f/f}$;THCre* AMs were compared with AMs from mice with adult-onset *Phd2* inactivation in all (*RosaCreER*) or only TH$^+$ (*THCreER*) cells. HIF isoform specificity of the observed phenotype in *Phd2$^{f/f}$;THCre* mice was tested by concomitant inactivation of *Phd2* and *Hif-1α* or *Hif-2α* under the THCre driver.

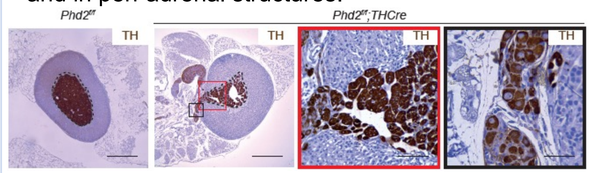
RESULTS

We report that **TH-restricted constitutive inactivation of *Phd2*** in the AM results in a **'pseudohypoxic pattern' of gene expression** in which dynamic activation of HIF transcription (in respect to target genes such as *Rgs5*) is superimposed on a **developmental shift in populations of AM cells** manifesting specific patterns of gene expression associated with the presence or absence of the noradrenaline-to-adrenaline converting enzyme phenylethanolamine *N*-methyltransferase (PNMT) which is acquired during final maturation of chromaffin cells. In detail, *Phd2* inactivation in the AM resulted in a striking **loss of *Pnmt*** mRNA.

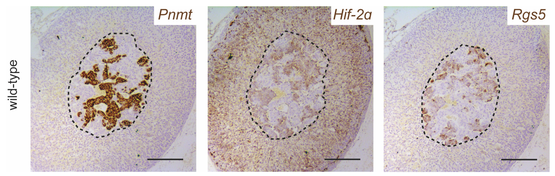


This finding correlated with a switch in plasma catecholamine profile with an **increase in noradrenaline** and a **decrease in adrenaline** levels, in line with clinical observations in patients with pseudohypoxic PCCs.

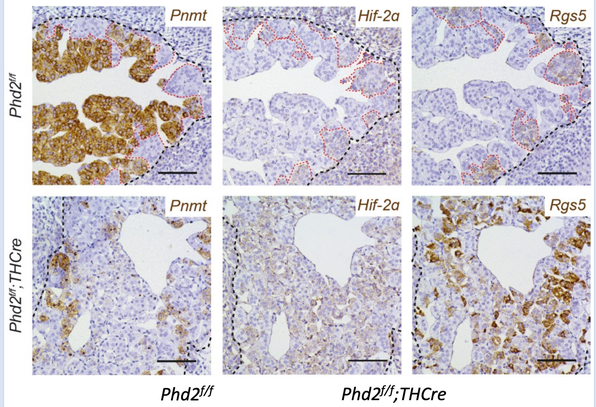
Although *Phd2$^{f/f}$;THCre* mice did not develop frank PCCs, changes in gene expression were accompanied by **morphological abnormalities** including ectopic TH$^+$ cells within the adrenal cortex and in peri-adrenal structures.

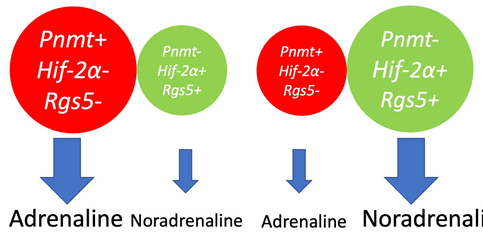


Analysis of spatial gene expression in wild-type AMs revealed a binary distribution of chromaffin cells with two contrasting patterns of expression: a **dominating population of differentiated *Pnmt*$^+$/*Hif-2α*^{low}/*Rgs5*^{low} cells** and a few clusters of **immature *Pnmt*$^-$/*Hif-2α*$^+$/*Rgs5*$^+$ cells**.



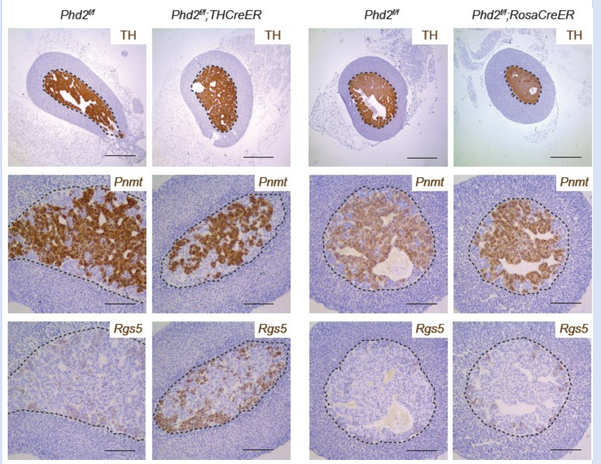
***Phd2* inactivation** resulted in a **switch of proportions** of these cell populations towards a **dominating immature phenotype**, explaining the overall loss of *Pnmt* expression and the dominant noradrenergic secretory profile in these mice.





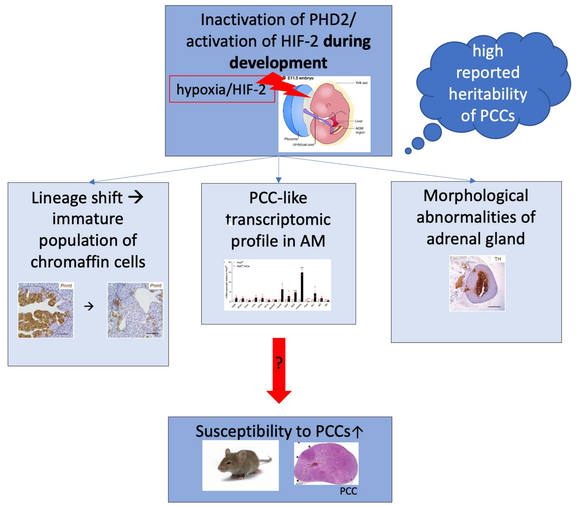
Both the morphological and gene expression alterations were ***Hif-2α***, but not *Hif-1α* dependent.

Interestingly, neither the **morphological abnormalities nor** a ***Pnmt* loss** could be observed with **adult-onset *Phd2* inactivation** although a slight induction of *Rgs5* was noted in *Pnmt*$^-$ areas, suggesting an effective dynamic activation of HIF transcriptional targets in these areas.



These findings suggest that the pathological activation of the PHD2/HIF-2 pathway during adrenal development is critical for its tumourigenic action.

CONCLUSIONS



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