HIF2 and endocrine neoplasia: an evolving story

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Abstract

In this issue of *Endocrine-Related Cancer*, Toledo *et al*. report the identification of activating mutations in the *HIF2* (*EPAS1*) transcription factor in a subset of sporadic pheochromocytomas and paragangliomas. These findings add significantly to an evolving and complex story of the role of hypoxic gene response pathways in human endocrine neoplasia.

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The heterodimeric HIF transcription factors regulate cellular responses to hypoxia. Each heterodimer consists of a specific α-subunit (HIF1α, HIF2α and HIF3α) which, in hypoxic conditions, complexes with the HIFβ (ARNT) subunit (Kaelin & Ratcliffe 2008). The best characterised factors, HIF1 and HIF2, have overlapping, but differing, roles in the hypoxic gene response. Thus, HIF1 and HIF2 regulate expression of ~200 genes implicated in angiogenesis, energy metabolism, cell proliferation and apoptosis, and while some targets are shared, others are preferentially regulated by HIF1 (BNIP3) or HIF2 (CCND1, VEGF) (Raval *et al*. 2005). Interestingly, in renal tumours, whereas HIF2 has been demonstrated to drive oncogenesis, loss-of-function mutations have been described in *HIF1A* and it has been suggested that HIF1 might have a tumour suppressor effect (Morris *et al*. 2009, Shen *et al*. 2011). HIF1α and HIF2α contain an N-terminal and a C-terminal transactivation domain (NTAD and CTAD). HIFβ is stably expressed but HIFα subunits are unstable and intracellular levels of HIF1 and HIF2 are primarily regulated by stabilisation of the HIFα subunits. Thus, in normoxic conditions, a family of prolyl hydroxylases modify two proline residues near the NTAD that enable the *VHL* tumour suppressor gene product (pVHL) (which acts as the substrate recognition subunit of an E3-ubiquitin ligase complex) to bind to HIFα and target it for degradation (Kaelin & Ratcliffe 2008). In hypoxia, the oxygen-dependent prolyl hydroxylases are inactive, pVHL-dependent degradation of HIFα subunits is compromised and HIF1 and HIF2 are stabilised and activate downstream transcriptional pathways.

A notable feature of pheochromocytoma and paraganglioma is the high incidence of inherited cases such that germline mutations in at least ten genes (e.g. *NF1*, *RET*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *TMEM127*, *MAX* and *VHL*) can be detected in more than one third of all cases (Gimenez-Roqueplo *et al*. 2012, Jafri & Maher 2012). Interestingly, inherited pheochromocytomas and paragangliomas can be subdivided by gene expression profiling studies according to the activity of hypoxic gene response pathways. Thus, activation of HIF-regulated pathways is seen in von Hippel–Lindau (VHL) disease and succinate dehydrogenase (SDH) subunit-associated tumours but not in pheochromocytomas and paragangliomas associated with *NF1*, *RET*, *TMEM127* and *MAX* mutations (Eisenhofer *et al*. 2004, Dahia *et al*. 2005, Pollard *et al*. 2006, Favier *et al*. 2009).

Sporadic pheochromocytomas and paragangliomas can also be subdivided according to hypoxic gene expression profiling with approximately half of tumours displaying a VHL/SDH-like profile (Dahia *et al*. 2005). Nevertheless, somatic inactivation of VHL- and SDH-subunit genes can

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be demonstrated in only a minority of sporadic cases, suggesting alternative mechanisms for activating hypoxic gene pathways (Eng *et al.* 1995, Astuti *et al.* 2004, Yao *et al.* 2010). Recently, several groups have demonstrated that oncogenic mutations in *HIF2A*/*EPAS1* leading to HIF2 stabilisation can be implicated in the pathogenesis of pheochromocytomas and paragangliomas (Zhuang *et al.* 2012, Comino-Méndez *et al.* 2013, Lorenzo *et al.* 2013, Taïeb *et al.* 2013, Toledo *et al.* 2013, Yang *et al.* 2013).

Initially, Zhuang *et al.* (2012) reported two unrelated patients who presented with polycythaemia and paragangliomas harbouring gain-of-function *HIF2A* mutations. Two different *HIF2A* missense substitutions (p.Ala530Thr and p.Ala530Val) affecting the same exon 12 residue were detected. Previously, germline *HIF2A* mutations (e.g. p.Pro534Leu, p.Gly537Arg, p.Met535Val, p.Gly537Trp, p.Asp539Glu) had been identified in patients with inherited erythrocytosis (Percy *et al.* 2008*a*,*b*, Furlow *et al.* 2009), and although the *HIF2A* mutations were not detected in normal tissues from the two patients described by Zhuang *et al.* (2012), the presence of polycythaemia and the respective mutations in multiple tumours from each patient (paragangliomas in one patient and a paraganglioma and somatostatinoma in the other) suggested that each of the individuals were likely to be mosaic for a *HIF2A* mutation that occurred post-zygotically. The p.Ala530 residue is adjacent to one of the two key proline residues (p.Pro531) whose hydroxylation status is critical for pVHL-mediated degradation of HIF2α and Zhuang *et al.* (2012) demonstrated that both mutations impaired prolyl hydroxylation and promoted HIF2α stabilisation. Subsequently, the same group reported two additional patients with polycythaemia, paragangliomas and somatostatinomas but novel *HIF2A* missense substitutions (p.Leu529Pro and p.Y532C) in tumour cells (Yang *et al.* 2013). In addition, Lorenzo *et al.* (2013) reported a germline activating *HIF2A* mutation (p.F374Y) (inherited from an apparently unaffected parent) in a patient with polycythaemia and paraganglioma.

Comino-Méndez *et al.* (2013) extended the clinical phenotype of tumour *HIF2A* mutations. Of seven patients with *HIF2A* mutation-positive tumours, three presented with congenital polycythaemia and multiple paragangliomas (p.Ala530Thr, p.Pro531Ser and p.Pro531Leu), one with multiple paragangliomas only (p.Ala530Val) and three with a single pheochromocytoma/paraganglioma (p.Ile533_Pro534del, p.Pro534_Asp536del and p.Asp 539Tyr). Following the detection of a somatic *HIF2A* mutation in six sporadic pheochromocytomas and paragangliomas analysed by exome sequencing, Toledo *et al.* (2013) found somatic *HIF2A* mutations in 2.3% (4/167) sporadic tumours but not in inherited cases. Three mutations affected codon 531 (p.Pro531Thr, p.Pro531Ser and p.Pro531Leu) and were shown to be associated with increased HIF2 stability (transcriptional profiles were available for two tumours and these clustered with the pseudohypoxic group that contained tumours associated with *VHL* and *SDHX* mutations).

In summary, recent studies of *HIF2A* mutations in pheochromocytoma and paraganglioma have provided novel insights into the role of hypoxic gene response pathways in the pathogenesis of endocrine neoplasia. To date, a number of common themes are emerging. First, most *HIF2A* mutations associated with pheochromocytoma and paraganglioma are somatic/mosaic and cluster in or around p.Pro531 resulting in HIF2 stabilisation producing gain-of-function effect. The clinical phenotype is variable, ranging from multiple tumours (paragangliomas or pheochromocytomas or paraganglioma and somatostatinoma) with or without polycythaemia to a single pheochromocytoma/paraganglioma. The role of HIF2 in endocrine neoplasia is now a hot research topic for research and much remains to be defined. From a clinical perspective, further information is required on the range of tumours that might be detected in patients who harbour constitutional or who are mosaic for *HIF2A* mutations – it would not be surprising if the phenotype expanded. A further key question is whether tumour *HIF2A* mutations will have implications for prognosis and treatment. Toledo *et al.* (2013) found evidence that *HIF2A* mutations at p.Pro531 promoted dedifferentiation of chromaffin cells and reduced the latency of tumour growth in nude mice assays. Malignant paragangliomas are known to be associated with germline *SDHB* mutations and although malignancy has yet to be reported in *HIF2A*-mutated tumours, the significance of *HIF2A* mutations for rate of tumour growth and risk of additional primary tumours in apparently sporadic cases needs to be determined. Although somatic *HIF2A* mutations may explain a subset of sporadic pheochromocytomas and paragangliomas with a pseudohypoxic gene expression profile, it would appear that additional, as yet uncharacterised, mechanisms must be implicated in other cases. Finally, although surgery is the mainstay of treatment for non-metastatic paragangliomas, much research is ongoing to develop anti-HIF therapies and it will be interesting to see whether, in the future, the finding of a *HIF2A* mutation in an endocrine tumour will have implications for clinical management.

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