

REVIEW

HIF Signaling Pathway in Pheochromocytoma and Other Neuroendocrine Tumors

I. JOCHMANOVÁ^{1,2}, T. ZELINKA³, J. WIDIMSKÝ Jr.³, K. PACAK²

¹First Department of Internal Medicine, Faculty of Medicine, P. J. Šafárik University, Košice, Slovakia, ²Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA, ³Third Department of Medicine, Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

Received March 20, 2014

Accepted March 26, 2014

Summary

Hypoxia-inducible factors (HIFs) are transcription factors controlling energy, iron metabolism, erythropoiesis, and development. Dysregulation of these proteins contributes to tumorigenesis and cancer progression. Recent findings revealed the important role of HIFs in the pathogenesis of neuroendocrine tumors, especially pheochromocytoma (PHEO) and paraganglioma (PGL). PHEOs and PGLs are catecholamine-producing tumors arising from sympathetic- or parasympathetic-derived chromaffin tissue. To date, eighteen PHEO/PGL susceptibility genes have been identified. Based on the main signaling pathways, PHEOs/PGLs have been divided into two clusters, pseudohypoxic cluster 1 and cluster 2, rich in kinase receptor signaling and protein translation pathways. Recent data suggest that both clusters are interconnected *via* the HIF signaling and its role in tumorigenesis is supported by newly described somatic and germline mutations in *HIF2A* gene in patients with PHEOs/PGLs associated with polycythemia, and in some of them also with somatostatinoma. Moreover, HIF α signaling has also been shown to be upregulated in neuroendocrine tumors other than PHEO/PGL. Some of these tumors are components of hereditary tumor syndromes which can be associated with PHEO/PGL, but also in ileal carcinoids or melanoma. HIF signaling appears to be one of the crucial players in tumorigenesis, which could suggest new therapeutic approaches for treatment of neuroendocrine tumors.

Key words

Pheochromocytoma • Paraganglioma • Hypoxia-inducible factor • Oxygen sensing • Therapy

Corresponding author

K. Pacak, Section on Medical Neuroendocrinology, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver NICHD, NIH, Building 10-CRC, 1E-3140, 10 Center Dr., Bethesda, MD, 20892-1109, USA. E-mail: karel@mail.nih.gov

Introduction

PHEOs and PGLs are rare neuroendocrine catecholamine producing tumors. PHEOs arise from chromaffin cells within the adrenal medulla whereas tumors arising from extra-adrenal sympathetic and parasympathetic paraganglia are called PGLs (Pacak *et al.* 2007, Pacak 2011). The majority of PHEOs/PGLs are benign tumors but metastasis can also occur, especially in patients with a specific genetic background (Brouwers *et al.* 2006, Ayala-Ramirez *et al.* 2011, Eisenhofer *et al.* 2012). In the past, it was postulated that 10 % of these tumors were hereditary, but recent studies show 30-40 % PHEOs/PGLs to be genetically inherited (Neumann *et al.* 2002, Karasek *et al.* 2012). Moreover, 10-24 % of apparently sporadic tumors were found to have somatic mutations (Burnichon *et al.* 2011, Welander *et al.* 2012, Crona *et al.* 2014).

In this review, we focus on the role of hypoxia-

inducible factor signaling in neuroendocrine tumor development.

Update on genetics of PHEO/PGL

To date, 18 genes predisposing to PHEO/PGL development have been identified (Table 1). The susceptibility genes include the *VHL* tumor suppressor gene, the *NF1* tumor suppressor gene, the *RET* proto-oncogene, the SDH complex subunits genes (*SDHA*, *SDHB*, *SDHC*, and *SDHD*), and the *SDHAF2* gene (Karasek *et al.* 2012, Vicha *et al.* 2013). The new PHEO/PGL susceptibility genes include *TMEM127*, *MAX*, *HIF2A* and, by anecdotal reports, *KIF1Bβ*, *PHD2/EGLN1*, *H-RAS*, *K-RAS*, *IDH*, *FH*, and *BAP1* genes (Hrascan *et al.* 2008, Ladrone *et al.* 2008, Schlisio *et al.* 2008, Gaal *et al.* 2010, Qin *et al.* 2010, Astuti *et al.* 2011, Comino-Mendez *et al.* 2011, 2013, Jiang and Dahia

2011, Wadt *et al.* 2012, Zhuang *et al.* 2012, Crona *et al.* 2013, Castro-Vega *et al.* 2014).

Two main clusters, based on the main signaling pathways, currently represent almost all hereditary PHEOs/PGLs: 1) cluster 1, a pseudohypoxic cluster, which is mainly represented by *VHL*, *SDHx* and, probably *HIF2A* and *FH* mutations, and 2) rich in kinase receptor signaling and protein translation pathways, cluster 2 is mainly composed of *NF1*, *RET*, *KIF1Bβ*, *TMEM127*, and *MAX* mutations (Dahia *et al.* 2005, Gimenez-Roqueplo *et al.* 2012, Vicha *et al.* 2013, Castro-Vega *et al.* 2014). Although cell signaling in these two clusters seems to be distinct, recent findings uniquely unify important existing connections between signaling pathways in both clusters. Not only that, the HIF signaling pathways appear to be a crucial, perhaps the most important player, in PHEO/PGL tumorigenesis.

Table 1. PHEO/PGL susceptibility genes and genotype-phenotype correlations.

Gene	Syndrome	Locus	Inheritance	Biochemical phenotype	Common PHEO/PGL sites	Malignant potential
<i>SDHA</i>	-	5p15	AD	Unknown	HNPGL/TAPGL	0-14 %
SDHB	PGL4	1p36.13	AD	MN, NMN, MTY, NS	TAPGL/HNPGL/adrenal	43 %
SDHC	PGL3	1q21	AD	MN, NMN, NS	HNPGL/TAPGL/adrenal	Low
SDHD	PGL1	11q23	AD PI	MN, NMN, MTY, NS	HNPGL/TAPGL/adrenal	5 %
SDHAF2	PGL2	11q13.1	AD PI	Unknown	HNPGL	Unknown
VHL	VHL	3p25-26	AD	NMN	adrenal/TAPGL/HNPGL	Low (<5 %)
NF1	NF1	17q11.2	AD	MN, NMN	Adrenal	11 %
RET	MEN	10q11.2	AD	MN, NMN	Adrenal	Low (<1-5 %)
MAX	-	14q23.3	AD PI	NMN and MN	Adrenal	10-25 %
TMEM127	-	2q11.2	AD	MN and NMN	Adrenal/TAPGL/HNPGL	Rare (4 %)
HIF2A	Pacak-Zhuang	2p21-p16	Somatic Germline*	NMN	TAPGL/adrenal	Unknown
H-RAS	NA	11p15.5	Somatic	Unknown	Adrenal	Unknown
K-RAS	NA	12p12.1	Somatic	Unknown	Adrenal	Unknown
IDH1	NA	2q33.3	Somatic	Unknown	Unknown (HNPGL?)	Unknown
KIF1Bβ	NA	1p36.2	Germline	Unknown	Unknown (Adrenal?)	Unknown
PHD2/EGLN1	NA	1q42.1	Germline	Unknown	Unknown (TAPGL?)	Unknown
FH	NA	1q43	Germline	Unknown	Adrenal/TAPGL	Unknown/potentially high
BAP1	NA	3p21.1	Germline	Unknown	Unknown (TAPGL?)	Unknown

*Presenting only with PHEO/PGL together with polycythemia; most likely different from the typical somatic type of *HIF2A* mutation. AD, autosomal dominant; HNPGL, head and neck paraganglioma; MN, metanephrine; MTY, metoxytyramine; NMN, normetanephrine; NS, nonsecreting; PGL, paraganglioma; PHEO, pheochromocytoma; PI, paternal inheritance; TAPGL, thoracic or abdominal paraganglioma.

Hypoxia-inducible factors (HIFs)

HIFs are transcription factors which are activated under hypoxic or pseudohypoxic conditions and mediate adaptive responses of cells to these states, an idea originally pioneered by Semenza (2011) then later further studied and advanced by others (Schofield and Ratcliffe 2004, Kaelin and Ratcliffe 2008). Hypoxia reflects the lack of sufficient oxygen supply (O_2 concentration drops below 21 %); pseudohypoxia is the state where oxygen is present in a sufficient amount in cells but cannot be processed and further utilized due to an alteration in oxygen-sensing pathways. HIFs are composed of stable, constitutively expressed β -subunit, and ubiquitously expressed, oxygen-sensitive α -subunits, consisting of three isoforms; HIF-1 α , HIF-2 α , and HIF-3 α (Schofield and Ratcliffe 2004, Kaelin and Ratcliffe 2008, Keith *et al.* 2012).

Under normoxic conditions, HIF-1 α and HIF-2 α are degraded *via* the ubiquitin-proteasome pathway, which is controlled by several key enzymatic reactions, the first including prolyl hydroxylation by PHDs (also called as the main O_2 sensors) and the second represented by subsequent bindings to the VHL protein (for a review, see Kaelin and Ratcliffe 2008, Koh and Powis 2012). When these enzymatic reactions occur, HIFs do not stabilize well and they are degraded quickly by the proteasome. Additional and less important negative regulation of HIF is mediated *via* the HIF-3 α 2 splice variant (IPAS), which forms inactive complexes with HIF-1 α , and also *via* the FIH1 that hydroxylates HIF on the asparaginyl residue of the C-terminal domain. FIH1 blocks HIF-1 α interaction using coactivators with proteasome ultimately breaking down the complex (Makino *et al.* 2001, Lando *et al.* 2002, Jang *et al.* 2005, Heikkilä *et al.* 2011).

Hypoxia or pseudohypoxia lead to HIF α stabilization and its heterodimerization with HIF- β , binding of coactivators, and the transactivation of HIF target genes, including those associated with angiogenesis, hematopoiesis, glycolysis, cell growth, and cell migration (Maxwell *et al.* 2001, Lau *et al.* 2007, Kaelin and Ratcliffe 2008).

HIF-1 α and HIF-2 α are the best studied isoforms and were found to have mostly complementary, but also opposite functions (Hu *et al.* 2003, Carroll and Ashcroft 2006, Rankin *et al.* 2007). HIF-1 α is activated during short periods of severe hypoxia, while HIF-2 α is active under mild hypoxia for more prolonged periods of time

(Holmquist-Mengelbier *et al.* 2006). Term mild hypoxia is used for oxygen concentrations in tissues of 1-5 % and severe hypoxia is defined as below 1 % (Koh and Powis 2012). The distinct effect of different oxygen concentrations on HIF α activation is mediated by HAF, which marks HIF-1 α for degradation but transactivates HIF-2 α by binding to a different protein site than in HIF-1 α (Koh *et al.* 2008, 2011). Moreover, both HIF-1 α and HIF-2 α functionally interact with many other signal transduction and transcriptional systems, including NOTCH, WNT, and MYC pathway; thus, HIFs can also regulate gene expression *via* a hypoxia-independent mechanism (Kaelin and Ratcliffe 2008).

Distinct regulation of HIF α isoforms leads to distinct expression patterns of these transcription factors. HIF-1 α is expressed in all cells, whereas HIF-2 α is preferentially expressed in the endothelium, kidney, heart, lung, gastrointestinal epithelium, and neural crest cell derivatives, including chromaffin cells (Wiesener *et al.* 2003, Keith *et al.* 2012). HIF-2 α was shown to play an important role in the regulation of developmental processes of sympathoadrenal lineage; it also regulates catecholamine synthesis and secretion reflected by a typical noradrenergic phenotype (for a review, see Richter *et al.* 2013).

HIF signaling in tumorigenesis

HIF interaction with many signaling and transduction pathways and the presence of (pseudo)hypoxia in cancer lead to the presumption that HIF is one of the most important players in tumorigenesis. It was found that both HIF-1 α and HIF-2 α are overexpressed in most human cancers (Zhong *et al.* 1999, Talks *et al.* 2000, Hockel and Vaupel 2001). HIF-1 α and HIF-2 α expression in tumors vary among cell types and these differences most probably reflect the consequences of preferential HIF-1 α or HIF-2 α function in different cancer cell subtypes, varying stages of tumor progression (including a degree of hypoxia), and within microenvironmental changes of the tumor (Blouw *et al.* 2003, Raval *et al.* 2005, Semenza 2010, Keith *et al.* 2012). Although both HIF α subunits are often overexpressed in many tumors, HIF-2 α is preferentially linked to more aggressive lesions with a higher metastatic potential or already present metastases (Warnecke *et al.* 2004, Koh and Powis 2012).

Thus, HIF-2 α overexpression was found to be associated with metastatic progression and poor

prognosis in specific cancer types, especially astrocytoma, glioma, neuroblastoma, head and neck cancers, and melanoma (Keith *et al.* 2012). In renal cell carcinoma, in which VHL mutations lead to decreased HIF α degradation by the defective bindings of hydroxylated HIFs to pVHL, HIF-2 α stabilization leads to the tumor formation in mice (Kondo *et al.* 2003) and protumorigenic genes were shown to be more HIF-2 α dependent (Raval *et al.* 2005). However, it was also shown that both HIF-2 α upregulation and downregulation can promote tumor development and progression (Kim *et al.* 2009, Mazumdar *et al.* 2010) therefore, a certain balance between HIF-1 α and HIF-2 α is needed for optimal function, specifically in tumors. Moreover, HIF-2 α has been shown to regulate HIF-1 α target genes expression in the absence of HIF-1 α and vice versa (Keith *et al.* 2012, Koh and Powis 2012). Thus, it is not surprising that renal cell carcinomas may be divided into two major subgroups; those preferentially expressing HIF-2 α and those with combined expression of HIF-1 α and HIF-2 α .

HIF activation in cancer is mediated not only *via* the (pseudo)hypoxia signaling pathway but also *via* multiple oxygen-independent oncogenic pathways, including growth factor signaling pathways and a loss of tumor suppressor genes (for a review, see Jochmanova *et al.* 2013, Richter *et al.* 2013).

HIF in PHEO/PGL development

The role of HIF in the PHEO/PGL pathogenesis has been anticipated for a long time since HIF pathway was previously found to be dysfunctional in these tumors (Maxwell *et al.* 2001, Dahia *et al.* 2005, Favier and Gimenez-Roqueplo 2010). Interestingly, most of hereditary PHEOs/PGLs are indeed related to the hypoxia signaling pathway, mainly through the mutations within the *VHL*, *PHD2*, *FH*, *SDHx*, and *SDHAF2* genes (Jafri and Maher 2012, Toledo *et al.* 2013, Castro-Vega *et al.* 2014), which lead to increased stability of HIF (Gimenez-Roqueplo *et al.* 2001). In the *RET*- and *NF1*-associated PHEOs/PGLs, the activation of the RAS-MAPK-mTORC pathway is present, which leads to HIF-1 α activation (Eisenhofer *et al.* 2004a, Dahia *et al.* 2005, Johannessen *et al.* 2005, Foster and Fingar 2010, Lopez-Jimenez *et al.* 2010). In tumors associated with *TMEM127* and *MAX* mutations, HIF α levels seem to be increased also due to mTORC activation (Brugarolas *et al.* 2004, Land and Tee 2007; for a review, see Jochmanova *et al.* 2013). *K-RAS*

and recently described *H-RAS* mutations in PHEOs/PGLs lead to the activation of the RAS/RAF/ERK pathway, a part of the RAS/MAPK pathway (Hrascan *et al.* 2008, Crona *et al.* 2013), and to the increased HIF α signaling. *FH* mutations result in a loss of fumarate hydratase activity, an enzyme important in the Krebs cycle. *FH*-related PHEOs/PGLs had widespread alterations in protein succination and displayed the same epigenetic changes as *SDHB*-related tumors (Castro-Vega *et al.* 2014).

One of the most recently described genes associated with PHEO/PGL development is *HIF2A*. Initially, somatic gain-of-function *HIF2A* mutations were found in tumors from two female patients with multiple PGLs and polycythemia. One of these patients also presented with somatostatinoma (Zhuang *et al.* 2012). Subsequently, two other patients with multiple PGLs and somatostatins associated with polycythemia were also found to have somatic *HIF2A* mutations, suggesting the existence of a new syndrome described by Pacak and Zhuang (Pacak *et al.* 2013). *HIF2A* mutations result in HIF-2 α protein stabilization by affecting its hydroxylation, which prevents recognition of HIF-2 α by VHL and results in increased HIF-2 α protein half-life followed by changes in transcriptional activity of many hypoxia-related genes (Zhuang *et al.* 2012, Yang *et al.* 2013). Further investigations revealed other patients with PHEO and this syndrome, extending the clinical characteristics of the Pacak-Zhuang syndrome to multiple PHEOs (Taieb *et al.* 2013). Subsequently, Lorenzo *et al.* (2013) found a male patient presenting with PHEO and PGLs associated with polycythemia due to a germline *HIF2A* mutation and Favier *et al.* (2012) identified a *HIF2A* mutation in PHEO resected from a 24-year-old female without polycythemia. However, none of these presented with somatostatinoma. Comino-Méndez *et al.* (2013) described three additional female patients with multiple PHEOs/PGLs and polycythemia and three patients with apparently sporadic disease. The other patient with somatic *HIF2A* mutation – female presenting with polycythemia, PHEO, PGLs and somatostatinoma – has been described recently by Buffet *et al.* (2014). They also found a *HIF2A* heterozygous mutation in a male patient with polycythemia and PGL; the mutation was present in the PGL tissue but also as a mosaic in leukocyte DNA and DNA extracted from buccal cells. HIF-2 α stabilization and its involvement in the pathogenesis of PHEOs/PGLs is also supported by the presence of only the noradrenergic biochemical

phenotype, which reflects the involvement of HIF-2 α in preferential norepinephrine synthesis as described previously (Tian *et al.* 1998, Eisenhofer *et al.* 2004b, Nilsson *et al.* 2005). In summary, the presence of multiple PHEOs/PGLs and duodenal somatostatinomas associated with polycythemia currently seem to only be present in female patients.

Previously, dominantly inherited gain-of-function *HIF2A* mutations were found to be associated with congenital polycythemia (Percy *et al.* 2008, Prchal and Gordeuk 2008), which stresses the role of HIF-2 α in the regulation of erythropoiesis. HIF-2 α stabilization and PGL-associated erythropoietin production have been also found in patients with *PHD2* and *VHL* mutations (Ladroue *et al.* 2008, Capodimonti *et al.* 2012).

The role of HIF in PHEO/PGL pathogenesis is further supported by the gene expression profiling study, which revealed distinct differences in gene expression between both hereditary and sporadic norepinephrine- and epinephrine-producing PHEOs/PGLs including detection of genes involved in hypoxia-angiogenic pathways, especially *HIF2A* (Eisenhofer *et al.* 2004b). Later on, in the study of Dahia *et al.* (2005), cluster 1 tumors were found to display a gene signature of the activated hypoxia pathway, associated with enhanced angiogenesis and extracellular matrix processes, which are known to be HIF-1 α signaling dependent. Subsequent other gene expression profiling studies confirmed strong *HIF2A* expression in VHL and SDHx tumors (Lopez-Jimenez *et al.* 2010, Burnichon *et al.* 2011). Moreover, Lee *et al.* (2005) proposed that the PHEO/PGL mutations of *NF1*, *RET*, *VHL* and *SDHx* all act on the same signaling network, resulting in decreased apoptosis (also dependent on the HIF signaling pathway) during chromaffin cell development and tumor formation; additionally, cluster 2 gene mutations seem to enhance HIF-2 α signaling in cells (for a review, see Jochmanova *et al.* 2013, Richter *et al.* 2013).

HIF and other neuroendocrine tumors

The HIF α signaling has also been shown to be upregulated in neuroendocrine tumors other than PHEO/PGL. Some of these tumors are components of hereditary tumor syndromes which can be associated with PHEO/PGL, e.g. VHL disease caused by the mutation of *VHL* or MEN due to the mutations in the *RET* proto-oncogene. In MEN type 1, pituitary, parathyroid and pancreas tumors are present, while in MEN type 2, there

are medullary thyroid carcinomas, PHEO, and parathyroid tumors or, simply with mucosal neuromas (Gaztambide *et al.* 2013, Lodish 2013). Medullary thyroid cancer is rare and typically occurs as a part of MEN2. A recent study by Takacova *et al.* (2014) brought the experimental evidence for the crosstalk between RET and HIF-1.

Speisky *et al.* (2012) studied the expression of 52 genes in patients with VHL and sporadic pancreatic neuroendocrine tumors. They found upregulation of genes directly related to HIF signaling (*CA9*, *HIF2A*, *GLUT1*), angiogenesis, epithelial to mesenchymal transition, and metastasis in VHL pancreatic neuroendocrine tumors.

Tumor hypoxia and overexpression of HIF-1 α and HIF-2 α were also found in ileal carcinoids. HIF-2 α expression was significantly higher in distant metastasis compared to a primary tumor in the same patient. Immunohistochemical analysis demonstrated expression of VEGF and CA9. Expression profiling of hypoxic carcinoid cells revealed significant regulation of a large number of genes, including the chemokine receptor CXCR4 (Arvidsson *et al.* 2010).

It has also been demonstrated that hypoxia can induce NOTCH downstream signaling in several tumor cell lines. Accumulation of HIF-2 α promotes an aggressive phenotype with dedifferentiation and activation of NOTCH signaling in neuroblastoma cells (Jogi *et al.* 2004, Holmquist-Mengelbier *et al.* 2006, Sahlgren *et al.* 2008).

High expression levels of HIF-1 α , HIF-2 α and their target genes have been detected in melanoma cells and they were found to be related to a poor prognosis of patients (Zhong *et al.* 1999, Giatromanolaki *et al.* 2003, Valencak *et al.* 2009). Enhanced expression of HIFs, transcription of their target genes, and altered metabolism in melanoma cells during disease progression may promote malignant reprogramming and acquisition of stem cell-like properties and more aggressive and metastatic phenotypes, reviewed by Mimeault and Batra (2013).

Conclusions and future treatment options

HIF α signaling appears to be a central signaling pathway involved in the pathogenesis of hereditary PHEOs/PGLs, sporadic PHEOs/PGLs, and other neuroendocrine tumors. This designates HIF α as a very promising therapeutic target, especially for more

aggressive and metastatic tumors where the introduction of HIF-2 α is of the greatest interest at present. Nowadays, several agents affecting HIF-1 α signaling have been introduced, with various results depending on a cancer HIF α phenotype (Liu *et al.* 2014, for a review, see Melillo 2007a,b). Nevertheless, drugs selectively targeting the HIF-2 α signaling pathways have not yet been described but are currently under development (Rogers *et al.* 2013, Scheuermann *et al.* 2013). Moreover, it has been shown that HIF-1 α and HIF-2 α can activate different genes (Keith *et al.* 2012, for a review, see Koh and Powi 2012); therefore, the development of drugs targeting both HIF-1 α and HIF-2 α is of great interest. It was shown that HAF switches cells from HIF-1 α to HIF-2 α signaling; in light of that, one therapeutic approach could be to change the balance of HIF α isoforms by modulating HAF signaling in tumors (Koh *et al.* 2011). Further investigations are needed to elucidate the other signaling pathways involved in PHEO/PGL development and crosstalk within these pathways and HIF signaling pathway. The combination of two or three drugs, one of them targeting the HIF α signaling pathway and the other two regulating signaling pathways independently of HIFs, could potentially serve as a promising therapeutic strategy in combatting metastatic neuroendocrine tumors, especially PHEO/PGL.

Finding of novel diagnostic tests based on biomarkers (detected either biochemically or by molecular imaging) associated with hypoxia and altered metabolic pathways (by measurement of various metabolites, specifically within the Krebs cycle) should also help to select patients who are likely to respond to that specific type of therapy (e.g. to the HIF signaling inhibitors) and to personalize anti-cancer treatment. We predict that the introduction of novel HIF-related therapies will soon be integrated into medical practices

that are specialized in the treatment of neuroendocrine tumors.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Supported by RVO-VFN 64165 and by the Intramural Research Program of the National Institutes of Health, NICHD.

Abbreviations

BAP1, BRCA1-associated protein-1; CA9, carbonic anhydrase-IX; CXCR4, C-X-C chemokine receptor type 4; ERK, extracellular signal-regulated kinase; FH, fumarate hydratase; FIH1, factor inhibiting HIF; GLUT1, glucose transporter 1; HAF, hypoxia-associated factor; HIF, hypoxia-inducible factor; *HIF2A*, hypoxia-inducible factor 2-alpha gene; *H-RAS*, Harvey rat sarcoma viral oncogene; IPAS, inhibitory PAS domain protein; IDH, isocitrate dehydrogenase; KIF1B β , kinesin family member 1B, transcript variant β ; *K-RAS*, Kirsten rat sarcoma viral oncogene; MAPK, mitogen activated protein kinases; MAX, MYC-associated factor X; MEN, multiple endocrine neoplasia; mTORC, mammalian target of rapamycin complex; MYC, v-myc avian myelocytomatosis viral oncogene homolog; NF1, neurofibromatosis type 1; PGL, paraganglioma; PHD, prolyl hydroxylase; PHD2/EGLN1, prolyl hydroxylase 2; PHEO, pheochromocytoma; pVHL, von Hippel-Lindau protein; RAF, proto-oncogene serine/threonine-protein kinase; RET, rearranged during transfection; SDH, succinate dehydrogenase; SDHAF2, SDH complex assembly factor 2; TMEM127, transmembrane protein 127; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.

References

- ARVIDSSON Y, BERGSTROM A, ARVIDSSON L, KRISTIANSSON E, AHLMAN H, NILSSON O: Hypoxia stimulates CXCR4 signalling in ileal carcinoids. *Endocr Relat Cancer* **17**: 303-316, 2010.
- ASTUTI D, RICKETTS CJ, CHOWDHURY R, McDONOUGH MA, GENTLE D, KIRBY G, SCHLISIO S, KENCHAPPA RS, CARTER BD, KAELIN WG JR, RATCLIFFE PJ, SCHOFIELD CJ, LATIF F, MAHER ER: Mutation analysis of HIF prolyl hydroxylases (PHD/EGLN) in individuals with features of pheochromocytoma and renal cell carcinoma susceptibility. *Endocr Relat Cancer* **18**: 73-83, 2011.
- AYALA-RAMIREZ M, FENG L, JOHNSON MM, EJAZ S, HABRA MA, RICH T, BUSAIDY N, COTE GJ, PERRIER N, PHAN A, PATEL S, WAGUESPACK S, JIMENEZ C: Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metab* **96**: 717-725, 2011.

- BLOUW B, SONG H, TIHAN T, BOSZE J, FERRARA N, GERBER HP, JOHNSON RS, BERGERS G: The hypoxic response of tumors is dependent on their microenvironment. *Cancer Cell* **4**: 133-146, 2003.
- BROUWERS FM, EISENHOFER G, TAO JJ, KANT JA, ADAMS KT, LINEHAN WM, PACAK K: High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. *J Clin Endocrinol Metab* **91**: 4505-4509, 2006.
- BRUGAROLAS J, LEI K, HURLEY RL, MANNING BD, REILING JH, HAFEN E, WITTERS LA, ELLISEN LW, KAELIN WG JR: Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* **18**: 2893-2904, 2004.
- BUFFET A, SMATI S, MANSUY L, MENARA M, LEBRAS M, HEYMANN MF, SIMIAN C, FAVIER J, MURAT A, CARIOU B, GIMENEZ-ROQUEPLO AP: Mosaicism in HIF2A-related polycythemia-paraganglioma syndrome. *J Clin Endocrinol Metab* **99**: E369-E373, 2014.
- BURNICHON N, VESCOVO L, AMAR L, LIBE R, DE REYNIES A, VENISSE A, JOUANNO E, LAURENDEAU I, PARFAIT B, BERTHERAT J, PLOUIN PF, JEUNEMAITRE X, FAVIER J, GIMENEZ-ROQUEPLO AP: Integrative genomic analysis reveals somatic mutations in pheochromocytoma and paraganglioma. *Hum Mol Genet* **20**: 3974-3985, 2011.
- CAPODIMONTI S, TEOFILI L, MARTINI M, CENCI T, IACHININOTO MG, NUZZOLO ER, BIANCHI M, MURDOLO M, LEONE G, LAROCCA LM: Von Hippel-Lindau disease and erythrocytosis. *J Clin Oncol* **30**: e137-e139, 2012.
- CARROLL VA, ASHCROFT M: Role of hypoxia-inducible factor (HIF)-1alpha versus HIF-2alpha in the regulation of HIF target genes in response to hypoxia, insulin-like growth factor-I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. *Cancer Res* **66**: 6264-6270, 2006.
- CASTRO-VEGA LJ, BUFFET A, DE CUBAS AA, CASCON A, MENARA M, KHALIFA E, AMAR L, AZRIEL S, BOURDEAU I, CHABRE O, CURRAS-FREIXES M, FRANCO-VIDAL V, GUILLAUD-BATAILLE M, SIMIAN C, MORIN A, LETON R, GOMEZ-GRANA A, POLLARD PJ, RUSTIN P, ROBLEDO M, FAVIER J, GIMENEZ-ROQUEPLO AP: Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum Mol Genet* **23**: 2440-2446, 2014.
- COMINO-MENDEZ I, GRACIA-AZNAREZ FJ, SCHIAVI F, LANDA I, LEANDRO-GARCIA LJ, LETON R, HONRADO E, RAMOS-MEDINA R, CARONIA D, PITA G, GOMEZ-GRANA A, DE CUBAS AA, INGLADA-PEREZ L, MALISZEWSKA A, TASCHIN E, BOBISSE S, PICA G, LOLI P, HERNANDEZ-LAVADO R, DIAZ JA, GOMEZ-MORALES M, GONZALEZ-NEIRA A, RONCADOR G, RODRIGUEZ-ANTONA C, BENITEZ J, MANNELLI M, OPOCHER G, ROBLEDO M, CASCON A: Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat Genet* **43**: 663-667, 2011.
- COMINO-MENDEZ I, DE CUBAS AA, BERNAL C, ALVAREZ-ESCOLA C, SANCHEZ-MALO C, RAMIREZ-TORTOSA CL, PEDRINACI S, RAPIZZI E, ERCOLINO T, BERNINI G, BACCA A, LETON R, PITA G, ALONSO MR, LEANDRO-GARCIA LJ, GOMEZ-GRANA A, INGLADA-PEREZ L, MANCIKOVA V, RODRIGUEZ-ANTONA C, MANNELLI M, ROBLEDO M, CASCON A: Tumoral EPAS1 (HIF2A) mutations explain sporadic pheochromocytoma and paraganglioma in the absence of erythrocytosis. *Hum Mol Genet* **22**: 2169-2176, 2013.
- CRONA J, DELGADO VERDUGO A, MAHARJAN R, STALBERG P, GRANBERG D, HELLMAN P, BJORKLUND P: Somatic mutations in H-RAS in sporadic pheochromocytoma and paraganglioma identified by exome sequencing. *J Clin Endocrinol Metab* **98**: E1266-E1271, 2013.
- CRONA J, NORDLING M, MAHARJAN R, GRANBERG D, STALBERG P, HELLMAN P, BJORKLUND P: Integrative genetic characterization and phenotype correlations in pheochromocytoma and paraganglioma tumours. *PLoS One* **9**: e86756, 2014.
- DAHIA PL, ROSS KN, WRIGHT ME, HAYASHIDA CY, SANTAGATA S, BARONTINI M, KUNG AL, SANSON G, POWERS JF, TISCHLER AS, HODIN R, HEITRITTER S, MOORE F, DLUHY R, SOSA JA, OCAL IT, BENN DE, MARSH DJ, ROBINSON BG, SCHNEIDER K, GARBER J, ARUM SM, KORBONITS M, GROSSMAN A, PIGNY P, TOLEDO SP, NOSE V, LI C, STILES CD: A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS Genet* **1**: 72-80, 2005.

- EISENHOFER G, BORNSTEIN SR, BROUWERS FM, CHEUNG NK, DAHIA PL, DE KRIJGER RR, GIORDANO TJ, GREENE LA, GOLDSTEIN DS, LEHNERT H, MANGER WM, MARIS JM, NEUMANN HP, PACAK K, SHULKIN BL, SMITH DI, TISCHLER AS, YOUNG WF JR: Malignant pheochromocytoma: current status and initiatives for future progress. *Endocr Relat Cancer* **11**: 423-436, 2004a.
- EISENHOFER G, HUYNH TT, PACAK K, BROUWERS FM, WALTHER MM, LINEHAN WM, MUNSON PJ, MANNELLI M, GOLDSTEIN DS, ELKAHLOUN AG: Distinct gene expression profiles in norepinephrine- and epinephrine-producing hereditary and sporadic pheochromocytomas: activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. *Endocr Relat Cancer* **11**: 897-911, 2004b.
- EISENHOFER G, LENDERS JW, SIEGERT G, BORNSTEIN SR, FRIBERG P, MILOSEVIC D, MANNELLI M, LINEHAN WM, ADAMS K, TIMMERS HJ, PACAK K: Plasma methoxytyramine: a novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumour size, location and SDHB mutation status. *Eur J Cancer* **48**: 1739-1749, 2012.
- FAVIER J, BUFFET A, GIMENEZ-ROQUEPLO AP: HIF2A mutations in paraganglioma with polycythemia. *N Engl J Med* **367**: 2161; author reply 2161-2162, 2012.
- FAVIER J, GIMENEZ-ROQUEPLO AP: Pheochromocytomas: the (pseudo)-hypoxia hypothesis. *Best Pract Res Clin Endocrinol Metab* **24**: 957-968, 2010.
- FOSTER KG, FINGAR DC: Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. *J Biol Chem* **285**: 14071-14077, 2010.
- GAAL J, BURNICHON N, KORPERSHOEK E, RONCELIN I, BERTHERAT J, PLOUIN PF, DE KRIJGER RR, GIMENEZ-ROQUEPLO AP, DINJENS WN: Isocitrate dehydrogenase mutations are rare in pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab* **95**: 1274-1278, 2010.
- GAZTAMBIDE S, VAZQUEZ F, CASTANO L: Diagnosis and treatment of multiple endocrine neoplasia type 1 (MEN1). *Minerva Endocrinol* **38**: 17-28, 2013.
- GIATROMANOLAKI A, SIVRIDIS E, KOUSKOUKIS C, GATTER KC, HARRIS AL, KOUKOURAKIS MI: Hypoxia-inducible factors 1alpha and 2alpha are related to vascular endothelial growth factor expression and a poorer prognosis in nodular malignant melanomas of the skin. *Melanoma Res* **13**: 493-501, 2003.
- GIMENEZ-ROQUEPLO AP, DAHIA PL, ROBLEDO M: An update on the genetics of paraganglioma, pheochromocytoma, and associated hereditary syndromes. *Horm Metab Res* **44**: 328-333, 2012.
- GIMENEZ-ROQUEPLO AP, FAVIER J, RUSTIN P, MOURAD JJ, PLOUIN PF, CORVOL P, ROTIG A, JEUNEMAITRE X: The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. *Am J Hum Genet* **69**: 1186-1197, 2001.
- HEIKKILA M, PASANEN A, KIVIRIKKO KI, MYLLYHARJU J: Roles of the human hypoxia-inducible factor (HIF)-3alpha variants in the hypoxia response. *Cell Mol Life Sci* **68**: 3885-3901, 2011.
- HOCKEL M, VAUPEL P: Biological consequences of tumor hypoxia. *Semin Oncol* **28**: 36-41, 2001.
- HOLMQUIST-MENGELBIER L, FREDLUND E, LOFSTEDT T, NOGUERA R, NAVARRO S, NILSSON H, PIETRAS A, VALLON-CHRISTERSSON J, BORG A, GRADIN K, POELLINGER L, PAHLMAN S: Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. *Cancer Cell* **10**: 413-423, 2006.
- HRASCAN R, PECINA-SLAUS N, MARTIC TN, COLIC JF, GALL-TROSELJ K, PAVELIC K, KARAPANDZA N: Analysis of selected genes in neuroendocrine tumours: insulinomas and phaeochromocytomas. *J Neuroendocrinol* **20**: 1015-1022, 2008.
- HU CJ, WANG LY, CHODOSH LA, KEITH B, SIMON MC: Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. *Mol Cell Biol* **23**: 9361-9374, 2003.
- JAFRI M, MAHER ER: The genetics of phaeochromocytoma: using clinical features to guide genetic testing. *Eur J Endocrinol* **166**: 151-158, 2012.
- JANG MS, PARK JE, LEE JA, PARK SG, MYUNG PK, LEE DH, PARK BC, CHO S: Binding and regulation of hypoxia-inducible factor-1 by the inhibitory PAS proteins. *Biochem Biophys Res Commun* **337**: 209-215, 2005.
- JIANG S, DAHIA PL: Minireview: the busy road to pheochromocytomas and paragangliomas has a new member, TMEM127. *Endocrinology* **152**: 2133-2140, 2011.

- JOGI A, VALLON-CHRISTERSSON J, HOLMQUIST L, AXELSON H, BORG A, PAHLMAN S: Human neuroblastoma cells exposed to hypoxia: induction of genes associated with growth, survival, and aggressive behavior. *Exp Cell Res* **295**: 469-487, 2004.
- JOHANNESSEN CM, RECZEK EE, JAMES MF, BREMS H, LEGIUS E, CICHOWSKI K: The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci USA* **102**: 8573-8578, 2005.
- JOCHMANOVA I, YANG C, ZHUANG Z, PACAK K: Hypoxia-inducible factor signaling in pheochromocytoma: turning the rudder in the right direction. *J Natl Cancer Inst* **105**: 1270-1283, 2013.
- KAELIN WG JR, RATCLIFFE PJ: Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* **30**: 393-402, 2008.
- KARASEK D, SHAH U, FRYSAK Z, STRATAKIS CA, PACAK K: An update on the genetics of pheochromocytoma. *J Hum Hypertens* **27**: 141-147, 2012.
- KEITH B, JOHNSON RS, SIMON MC: HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* **12**: 9-22, 2012.
- KIM WY, PERERA S, ZHOU B, CARRETERO J, YEH JJ, HEATHCOTE SA, JACKSON AL, NIKOLINAKOS P, OSPINA B, NAUMOV G, BRANDSTETTER KA, WEIGMAN VJ, ZAGHLUL S, HAYES DN, PADERA RF, HEYMACH JV, KUNG AL, SHARPLESS NE, KAELIN WG JR, WONG KK: HIF2alpha cooperates with RAS to promote lung tumorigenesis in mice. *J Clin Invest* **119**: 2160-2170, 2009.
- KOH MY, DARNAY BG, POWIS G: Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1alpha, leading to its oxygen-independent degradation. *Mol Cell Biol* **28**: 7081-7095, 2008.
- KOH MY, LEMOS R JR, LIU X, POWIS G: The hypoxia-associated factor switches cells from HIF-1alpha- to HIF-2alpha-dependent signaling promoting stem cell characteristics, aggressive tumor growth and invasion. *Cancer Res* **71**: 4015-4027, 2011.
- KOH MY, POWIS G: Passing the baton: the HIF switch. *Trends Biochem Sci* **37**: 364-372, 2012.
- KONDO K, KIM WY, LECHPAMMER M, KAELIN WG JR: Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. *PLoS Biol* **1**: E83, 2003.
- LADROUE C, CARCENAC R, LEPORRIER M, GAD S, LE HELLO C, GALATEAU-SALLE F, FEUNTEUN J, POUYSSEGUR J, RICHARD S, GARDIE B: PHD2 mutation and congenital erythrocytosis with paraganglioma. *N Engl J Med* **359**: 2685-2692, 2008.
- LAND SC, TEE AR: Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *J Biol Chem* **282**: 20534-20543, 2007.
- LANDO D, PEET DJ, GORMAN JJ, WHELAN DA, WHITELAW ML, BRUICK RK: FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* **16**: 1466-1471, 2002.
- LAU KW, TIAN YM, RAVAL RR, RATCLIFFE PJ, PUGH CW: Target gene selectivity of hypoxia-inducible factor-alpha in renal cancer cells is conveyed by post-DNA-binding mechanisms. *Br J Cancer* **96**: 1284-1292, 2007.
- LEE S, NAKAMURA E, YANG H, WEI W, LINGGI MS, SAJAN MP, FARESE RV, FREEMAN RS, CARTER BD, KAELIN WG JR, SCHLISIO S: Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer Cell* **8**: 155-167, 2005.
- LIU XW, CAI TY, ZHU H, CAO J, SU Y, HU YZ, HE QJ, YANG B: Q6, a novel hypoxia-targeted drug, regulates hypoxia-inducible factor signaling via an autophagy-dependent mechanism in hepatocellular carcinoma. *Autophagy* **10**: 111-122, 2014.
- LODISH M: Multiple endocrine neoplasia type 2. *Front Horm Res* **41**: 16-29, 2013.
- LOPEZ-JIMENEZ E, GOMEZ-LOPEZ G, LEANDRO-GARCIA LJ, MUNOZ I, SCHIAVI F, MONTERO-CONDE C, DE CUBAS AA, RAMIRES R, LANDA I, LESKELA S, MALISZEWSKA A, INGLADA-PEREZ L, DE LA VEGA L, RODRIGUEZ-ANTONA C, LETON R, BERNAL C, DE CAMPOS JM, DIEZ-TASCON C, FRAGA MF, BOULLOSA C, PISANO DG, OPOCHER G, ROBLEDO M, CASCON A: Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Mol Endocrinol* **24**: 2382-2391, 2010.

- LORENZO FR, YANG C, NG TANG FUI M, VANKAYALAPATI H, ZHUANG Z, HUYNH T, GROSSMANN M, PACAK K, PRCHAL JT: A novel EPAS1/HIF2A germline mutation in a congenital polycythemia with paraganglioma. *J Mol Med (Berl)* **91**: 507-512, 2013.
- MAKINO Y, CAO R, SVENSSON K, BERTILSSON G, ASMAN M, TANAKA H, CAO Y, BERKENSTAM A, POELLINGER L: Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* **414**: 550-554, 2001.
- MAXWELL PH, PUGH CW, RATCLIFFE PJ: Activation of the HIF pathway in cancer. *Curr Opin Genet Dev* **11**: 293-299, 2001.
- MAZUMDAR J, HICKEY MM, PANT DK, DURHAM AC, SWEET-CORDERO A, VACHANI A, JACKS T, CHODOSH LA, KISSIL JL, SIMON MC, KEITH B: HIF-2alpha deletion promotes Kras-driven lung tumor development. *Proc Natl Acad Sci USA* **107**: 14182-14187, 2010.
- MELILLO G: Hypoxia-inducible factor 1 inhibitors. *Methods Enzymol* **435**: 385-402, 2007a.
- MELILLO G: Targeting hypoxia cell signaling for cancer therapy. *Cancer Metastasis Rev* **26**: 341-352, 2007b.
- MIMEAULT M, BATRA SK: Hypoxia-inducing factors as master regulators of stemness properties and altered metabolism of cancer- and metastasis-initiating cells. *J Cell Mol Med* **17**: 30-54, 2013.
- NEUMANN HPH, BAUSCH B, MCWHINNEY SR, BENDER BU, GIMM O, FRANKE G, SCHIPPER J, KLISCH J, ALTEHOEFER C, ZERRES K, JANUSZEWICZ A, ENG C: Germ-line mutations in nonsyndromic pheochromocytoma. *New Engl J Med* **346**: 1459-1466, 2002.
- NILSSON H, JOGI A, BECKMAN S, HARRIS AL, POELLINGER L, PAHLMAN S: HIF-2alpha expression in human fetal paraganglia and neuroblastoma: relation to sympathetic differentiation, glucose deficiency, and hypoxia. *Exp Cell Res* **303**: 447-456, 2005.
- PACAK K: Pheochromocytoma: a catecholamine and oxidative stress disorder. *Endocr Regul* **45**: 65-90, 2011.
- PACAK K, EISENHOFER G, AHLMAN H, BORNSTEIN SR, GIMENEZ-ROQUEPLO AP, GROSSMAN AB, KIMURA N, MANNELLI M, MCNICOL AM, TISCHLER AS: Pheochromocytoma: recommendations for clinical practice from the First International Symposium. *Nat Clin Pract Endocrinol Metab* **3**: 92-102, 2007.
- PACAK K, JOCHMANOVA I, PRODANOV T, YANG C, MERINO MJ, FOJO T, PRCHAL JT, TISCHLER AS, LECHAN RM, ZHUANG Z: New syndrome of paraganglioma and somatostatinoma associated with polycythemia. *J Clin Oncol* **31**: 1690-1698, 2013.
- PERCY MJ, FURLOW PW, LUCAS GS, LI X, LAPPIN TR, McMULLIN MF, LEE FS: A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. *N Engl J Med* **358**: 162-168, 2008.
- PRCHAL JT, GORDEUK VR: The HIF2A gene in familial erythrocytosis. *N Engl J Med* **358**: 1966; author reply 1966-1967, 2008.
- QIN Y, YAO L, KING EE, BUDDAVARAPU K, LENCI RE, CHOCRON ES, LECHLEITER JD, SASS M, ARONIN N, SCHIAVI F, BOARETTO F, OPOCHER G, TOLEDO RA, TOLEDO SP, STILES C, AGUIAR RC, DAHIA PL: Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat Genet* **42**: 229-233, 2010.
- RANKIN EB, BIJU MP, LIU Q, UNGER TL, RHA J, JOHNSON RS, SIMON MC, KEITH B, HAASE VH: Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. *J Clin Invest* **117**: 1068-1077, 2007.
- RAVAL RR, LAU KW, TRAN MG, SOWTER HM, MANDRIOTA SJ, LI JL, PUGH CW, MAXWELL PH, HARRIS AL, RATCLIFFE PJ: Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* **25**: 5675-5686, 2005.
- RICHTER S, QIN N, PACAK K, EISENHOFER G: Role of hypoxia and HIF2alpha in development of the sympathoadrenal cell lineage and chromaffin cell tumors with distinct catecholamine phenotypic features. *Adv Pharmacol* **68**: 285-317, 2013.
- ROGERS JL, BAYEH L, SCHEUERMANN TH, LONGGOOD J, KEY J, NAIDOO J, MELITO L, SHOKRI C, FRANTZ DE, BRUICK RK, GARDNER KH, MACMILLAN JB, TAMBAR UK: Development of inhibitors of the PAS-B domain of the HIF-2alpha transcription factor. *J Med Chem* **56**: 1739-1747, 2013.
- SAHLGREN C, GUSTAFSSON MV, JIN S, POELLINGER L, LENDAHL U: Notch signaling mediates hypoxia-induced tumor cell migration and invasion. *Proc Natl Acad Sci USA* **105**: 6392-6397, 2008.

- SCHEUERMANN TH, LI Q, MA HW, KEY J, ZHANG L, CHEN R, GARCIA JA, NAIDOO J, LONGGOOD J, FRANTZ DE, TAMBAR UK, GARDNER KH, BRUICK RK: Allosteric inhibition of hypoxia inducible factor-2 with small molecules. *Nat Chem Biol* **9**: 271-279, 2013.
- SCHLISIO S, KENCHAPPA RS, VREDEVELD LC, GEORGE RE, STEWART R, GREULICH H, SHAHRIARI K, NGUYEN NV, PIGNY P, DAHIA PL, POMEROY SL, MARIS JM, LOOK AT, MEYERSON M, PEEPER DS, CARTER BD, KAELIN WG JR: The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. *Genes Dev* **22**: 884-893, 2008.
- SCHOFIELD CJ, RATCLIFFE PJ: Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* **5**: 343-354, 2004.
- SEMENZA GL: Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* **29**: 625-634, 2010.
- SEMENZA GL: Oxygen sensing, homeostasis, and disease. *N Engl J Med* **365**: 537-547, 2011. Erratum in *N Engl J Med* **365**: 968, 2011.
- SPEISKY D, DUCES A, BIECHE I, REBOURS V, HAMMEL P, SAUVANET A, RICHARD S, BEDOSSA P, VIDAUD M, MURAT A, NICCOLI P, SCOAZEC JY, RUSZNIEWSKI P, COUVELARD A: Molecular profiling of pancreatic neuroendocrine tumors in sporadic and Von Hippel-Lindau patients. *Clin Cancer Res* **18**: 2838-2849, 2012.
- TAIEB D, YANG C, DELENNE B, ZHUANG Z, BARLIER A, SEBAG F, PACAK K: First report of bilateral pheochromocytoma in the clinical spectrum of *HIF2A*-related polycythemia-paraganglioma syndrome. *J Clin Endocrinol Metab* **98**: E908-E913, 2013.
- TAKACOVA M, BULLOVA P, SIMKO V, SKVARKOVA L, POTURNAJOVA M, FEKETOVA L, BABAL P, KIVELA AJ, KUOPIO T, KOPACEK J, PASTOREK J, PARKKILA S, PASTOREKOVA S: Expression pattern of carbonic anhydrase ix in medullary thyroid carcinoma supports a role for RET-mediated activation of the HIF pathway. *Am J Pathol* **184**: 953-965, 2014.
- TALKS KL, TURLEY H, GATTER KC, MAXWELL PH, PUGH CW, RATCLIFFE PJ, HARRIS AL: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* **157**: 411-421, 2000.
- TIAN H, HAMMER RE, MATSUMOTO AM, RUSSELL DW, McKNIGHT SL: The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev* **12**: 3320-3324, 1998.
- TOLEDO RA, QIN Y, SRIKANTAN S, MORALES NP, LI Q, DENG Y, KIM SW, PEREIRA MA, TOLEDO SP, SU X, AGUIAR RC, DAHIA PL: In vivo and in vitro oncogenic effects of HIF2A mutations in pheochromocytomas and paragangliomas. *Endocr Relat Cancer* **20**: 349-359, 2013.
- VALENCAK J, KITTLER H, SCHMID K, SCHREIBER M, RADERER M, GONZALEZ-INCHAURRAGA M, BIRNER P, PEHAMBERGER H: Prognostic relevance of hypoxia inducible factor-1alpha expression in patients with melanoma. *Clin Exp Dermatol* **34**: e962-e964, 2009.
- VICHA A, MUSIL Z, PACAK K: Genetics of pheochromocytoma and paraganglioma syndromes: new advances and future treatment options. *Curr Opin Endocrinol Diabetes Obes* **20**: 186-191, 2013.
- WADT K, CHOI J, CHUNG JY, KIILGAARD J, HEEGAARD S, DRZEWIECKI KT, TRENT JM, HEWITT SM, HAYWARD NK, GERDES AM, BROWN KM: A cryptic BAP1 splice mutation in a family with uveal and cutaneous melanoma, and paraganglioma. *Pigment Cell Melanoma Res* **25**: 815-818, 2012.
- WARNECKE C, ZABOROWSKA Z, KURRECK J, ERDMANN VA, FREI U, WIESENER M, ECKARDT KU: Differentiating the functional role of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2alpha target gene in Hep3B and Kelly cells. *FASEB J* **18**: 1462-1464, 2004.
- WELANDER J, LARSSON C, BACKDAHL M, HARENI N, SIVLER T, BRAUCKHOFF M, SODERKVIST P, GIMM O: Integrative genomics reveals frequent somatic NF1 mutations in sporadic pheochromocytomas. *Hum Mol Genet* **21**: 5406-5416, 2012.

-
- WIESENER MS, JURGENSEN JS, ROSENBERGER C, SCHOLZE CK, HORSTRUP JH, WARNECKE C, MANDRIOTA S, BECHMANN I, FREI UA, PUGH CW, RATCLIFFE PJ, BACHMANN S, MAXWELL PH, ECKARDT KU: Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *Faseb J* **17**: 271-273, 2003.
- YANG C, SUN MG, MATRO J, HUYNH TT, RAHIMPOUR S, PRCHAL JT, LECHAN R, LONSER R, PACAK K, ZHUANG Z: Novel HIF2A mutations disrupt oxygen sensing, leading to polycythemia, paragangliomas, and somatostatinomas. *Blood* **121**: 2563-2566, 2013.
- ZHONG H, DE MARZO AM, LAUGHNER E, LIM M, HILTON DA, ZAGZAG D, BUECHLER P, ISAACS WB, SEMENZA GL, SIMONS JW: Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res* **59**: 5830-5835, 1999.
- ZHUANG Z, YANG C, LORENZO F, MERINO M, FOJO T, KEBEBEW E, POPOVIC V, STRATAKIS CA, PRCHAL JT, PACAK K: Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. *N Engl J Med* **367**: 922-930, 2012.
-