15 YEARS OF PARAGANGLIOMA

Genetics and mechanism of pheochromocytoma–paraganglioma syndromes characterized by germline SDHB and SDHD mutations

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Abstract

Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine neoplasms that derive from small paraganglionic tissues which are located from skull base to the pelvic floor. Genetic predisposition plays an important role in development of PPGLs. Since the discovery of first mutations in the succinate dehydrogenase D (SDHD) gene, which encodes the smallest subunit of mitochondrial complex II (SDH), genetic studies have revealed a major role for mutations in SDH subunit genes, primarily in SDHB and SDHD, in predisposition to both familial and non-familial PPGLs. SDH-mutated PPGLs show robust expression of hypoxia induced genes, and genomic and histone hypermethylation. These effects occur in part through succinate-mediated inhibition of α-ketoglutarate-dependent dioxygenases. However, details of mechanisms by which SDH mutations activate hypoxic pathways and trigger subsequent neoplastic transformation remain poorly understood. Here, we present a brief review of the genetic and mechanistic aspects of SDH-mutated PPGLs.

Key Words
- pheochromocytoma
- neuroendocrine tumors
- neoplasia
- molecular genetics
- molecular biology

Introduction

Paragangliomas are neuroendocrine neoplasms that may arise from parasympathetic or sympathetic paraganglia. In general, those arising from parasympathetic paraganglia are non-secretory, associated with head and neck paraganglia and are usually referred to as head and neck paragangliomas (HNPGGL), or more specifically ‘carotid paraganglioma’, ‘jugulotympanic paraganglioma’, etc., rather than by older terms such as ‘chemodectoma’ or ‘glomus jugulare’. Paragangliomas arising from sympathetic nervous system paraganglia usually arise in the abdomen and thorax, and secrete catecholamines (Tischler 2008). Thus they are functionally and histologically similar to pheochromocytomas (in older literature they were often described as extra-adrenal pheochromocytomas). Occasionally HNPGL can also secrete catecholamines (Erickson et al. 2001).

Familial HNPGL was first described more than 80 years ago (Chase 1933), and 25 years ago evidence of autosomal dominant inheritance with parent of origin effects (tumors were only manifest after paternal transmission, Fig. 1) was reported (van der Mey et al. 1989). Ten years later, Baysal et al. (2000) reported the seminal finding that
familial HNPGL was associated with germline mutations in succinate dehydrogenase D (SDHD; PGL1 locus) and subsequently, SDHD mutations were also demonstrated to be associated with sporadic and familial pheochromocytoma (Gimm et al. 2000, Astuti et al. 2001a). SDHD encodes the D subunit of the SDH hetrotetrameric enzyme that, together with SDHC, anchors the SDH complex to the inner mitochondrial inner membrane. SDH has critical roles in the Krebs cycle and respiratory chain electron transport (as part of mitochondrial complex II, Fig. 2). The SDHB gene product, containing three iron–sulfur clusters, is part of the hydrophilic catalytic domain and binds to the SDHA gene product that contains a covalently attached flavin adenine dinucleotide (FAD) co-factor and the substrate binding site. SDHB and SDHD gene products bind to each other and attach the complex II holoenzyme to the mitochondrial inner membrane. Soon after the associations of SDHD mutations with human disease, germline SDHC (PGL3) mutations were reported to cause familial HNPGL (inherited as an autosomal dominant trait without parent-of-origin effects) (Niemann & Muller 2000) and germline SDHB (PGL4) mutations were found to cause inherited susceptibility to HNPGL and pheochromocytomas and paragangliomas (PPGL) (Astuti et al. 2001b). Subsequently germline mutations in the SDH-associated protein SDHAF2 were found to be a rare cause of HNPGL (Hao et al. 2009, Bayley et al. 2010) and SDHA mutations, initially reported in the context of an autosomal recessive juvenile encephalopathy (Bourgeron et al. 1995), were demonstrated to be a rare cause of (dominantly inherited) predisposition to PPGL (though penetrance appears to be very low) (Burnichon et al. 2010). Though germline mutations in SDHC have been shown to be associated on rare occasions with PPGL (Mannelli et al. 2007), these are much less frequent than SDHB and SDHD mutations. Genetic heterogeneity of PPGLs is further highlighted by identification of germline mutations in the VHL, RET, NF1, TMEM127, and MAX genes (Dahia 2014). Here we review our current knowledge of SDHB- and SDHD-related disorders.

Germline SDHB and SDHD mutations

Mutation spectrum

A comprehensive database of germline SDHB and SDHD mutations is maintained at http://chromium.liacs.nl/LOVD2/SDH/home.php (Bayley et al. 2005). A wide variety of intragenic mutations have been described and, more recently, single or multiple exon deletions (and, occasionally, intragenic duplications; McWhinney et al. 2004, Cascon et al. 2008, Neumann et al. 2009). A number of frequent SDHB and SDHD mutations were observed and these may result from a high mutation rate or to founder effects. Thus the relative frequency of some mutations can vary with geographical location. In the Netherlands, two major SDHD founder mutations have been identified (c.274G>T (p.Asp92Tyr) and c.416T>C (p.Leu139Pro)), and these account for >90% of SDHD mutation carriers.
(van Hulsteijn et al. 2012). A SDHD c.33C→A (p.Cys11X) founder mutation has been reported in central Europe (Poland; Peczkowska et al. 2008). The common SDHD c.242C>T (p.Pro81Leu) mutation has been reported as both a recurrent and a founder mutation (Baysal et al. 2002). In the Dutch population, SDHB founder mutations are less common. The most frequent (a splice site mutation c.423+1G) intragenic mutation was about 15 times less common than the SDHD c.274G>T (p.Asp92Tyr) founder mutation, and a founder SDHB exon 3 deletion has also been reported (Bayley et al. 2009a,b, Hensen et al. 2012). In Spain, SDHB founder mutations (exon 1 deletion and SDHB c.166_170delCCT-CA) have also been reported (Cascon et al. 2008, 2009).

Penetrance and genotype–phenotype correlations

A major difference between the clinical presentation of germline SDHB and SDHD mutations is the parent-of-origin effect with the latter. Apart from a few exceptional cases in which clinical disease has developed after maternal transmission of a SDHD mutation (Yeap et al. 2011), the risk of clinical disease after a maternal transmission appears to be extremely remote. Notably, the paraganglioma phenotype in such cases appears mild or atypical (e.g. no multi-focal tumors) indicating functional inequality of the two parental alleles in tumor pathogenesis (reviewed in Baysal (2013)). Though to date unequivocal evidence of genomic imprinting at the SDHD locus has not been found, paraganglia-specific partial (quantitative) imprinting of SDHD cannot be excluded. Differential methylation of a minor CpG island upstream of a long non-coding RNA located at the telomeric boundary of gene-rich SDHD domain was proposed to regulate long-range enhancer-promoter interactions (Baysal et al. 2011).

Homozygous SDHD mutations have been associated with recessively inherited encephalomyopathy and mitochondrial complex II deficiency (Jackson et al. 2014). Tumorigenesis in SDH-mutated neoplasia appears to follow a ‘two hit’ (retinoblastoma-like) model and it has been proposed that the parent-of-origin effects may reflect the tendency for the ‘second hit’ causing inactivation of the WT allele in SDHD-related tumorigenesis to be loss of the whole chromosome 11. The imprinted gene cluster at 11p15.5 contains the maternally expressed growth suppressor CDKN2B and the paternally expressed IGF2 growth factor (Lim & Maher 2010). In cases of a paternally inherited germline SDHD mutation, loss of the maternally-derived chromosome 11 would, in a single event, result in biallelic SDHD inactivation and loss of CDKN1C expression but preservation of IGF2 expression from the paternal allele (Hensen et al. 2004, Margetts et al. 2005). In contrast, it can be hypothesized that, in individuals harboring a maternally inherited SDHD mutation, loss of the paternally-derived chromosome 11 would, whilst biallelically inactivating SDHD, result in loss of IGF2 expression and retention of CDKN1C expression. Such a combination is not usually sufficient to drive tumorigenesis. In support of this hypothesis is the observation that in one case of paraganglioma after maternal transmission of a SDHD mutation, there was loss of the paternal SDHD allele and loss of the maternal 11p15.5 imprinted region (Yeap et al. 2011).

An alternative model to explain the parent-of-origin effects in transmission of SDHD-related paragangliomas suggests regulation of SDHD gene expression by a long-range epigenetic mechanism (Baysal et al. 2011). This model proposes that an imprinted small CpG island associated with a long intergenic non-coding RNA at the boundary of gene-rich SDHD domain regulates availability of a hypothetical distal enhancer to the SDHD promoter.

Particularly for germline SDHB mutations, the increased use of presymptomatic genetic testing in extended families has resulted in recognition that the penetrance of SDHB mutations is lower than initially thought. Thus initial estimates of the penetrance of germline SDHB mutations were in excess of 70% but have progressively fallen to 25–40% (Benn et al. 2006, Solis et al. 2009, Hes et al. 2010, Ricketts et al. 2010, Schiavi et al. 2010). The relatively low penetrance of SDHB mutations is consistent with the observation of a low de novo mutation rate, frequent founder mutations and the relatively high number of mutations detected in apparently isolated cases (Baysal et al. 2002, Neumann et al. 2002, Cascon et al. 2009, Jafri et al. 2013). However, the low penetrance can make the interpretation of likely pathogenicity for a novel sequence variant detected in individuals with a potentially SDH-related neoplasm complex and also raises, as yet unresolved questions, as to the type and intensity of tumor surveillance in asymptomatic gene carriers.

Though SDHB and SDHD encode components of the same protein complex, there are some differences in the relative propensities for developing different tumor types. Thus SDHD mutations are generally associated with a higher risk of HNPGL than non-HNPGL. For SDHB mutations, extra-adrenal and non-HNPGL is more often the presenting feature than HNPGL or pheochromocytoma, and there is a significantly higher risk of malignant paraganglioma and poor prognosis (~ 25% lifetime risk; Gimenez-Roqueplo et al. 2003, Amar et al. 2007, Ricketts et al. 2010). Despite the heterogeneity of SDHB mutations,
there are no clear genotype–phenotype correlations but for SDHD, though it has been suggested that the common p.Pro81Leu mutation is associated with a very low risk of PPGL (in contrast to truncating SDHD mutations for which the risk is closer to that seen with germline SDHB mutations; Ricketts et al. 2010).

A small number of additional tumor types have been reported in individuals with germline SDHB and SDHD mutations. Gastrointestinal tumors (GIST) are the best defined association. Carney–Stratakis syndrome is characterized by the association of GIST with paraganglioma and, in most cases is caused by mutations in SDHX genes (McWhinney et al. 2007, Janeway et al. 2011). Germline SDHX mutation may also be detected in patients with familial or sporadic nonsyndromic WT GIST (Janeway et al. 2011).

Renal tumors have been reported, predominantly with SDHB mutations, but also with SDHD/SDHC mutations and may be the presenting feature in patients without a personal or family history of HNPGL/PPGL (Vanharanta et al. 2004, Ricketts et al. 2008, 2010). A variety of histopathologies may occur (e.g. conventional (clear cell), papillary and oncocytoma) and the lifetime risk of renal tumors in SDHB mutation carriers has been estimated to be up to 15% (Ricketts et al. 2010).

Recently, a clinical association between pituitary adenoma and PPGL has been recognized. Molecular genetic studies have shown that this association may be caused by a variety of germline mutations in known PPGL predisposition genes (e.g. SDHB, SDHD, SDHC, VHL, and MEN1), or may be sporadic, but the most frequently implicated genes are SDH-subunit genes (Xekouki et al. 2011, Papathomas et al. 2013, Dénes et al. 2015).

**Application of SDHB and SDHD mutation testing in clinical practice**

The recognition that a substantial proportion (approximately one-quarter of apparently sporadic cases (Neumann et al. 2002)) might harbor a germline mutation in SDHB, SDHD, VHL, or RET led to suggestions that all PPGL patients might be offered genetic testing. However, such an approach, particularly prior to the application of next generation sequencing techniques, was expensive and did not take into account the how clinical indicators can be used. In particular, family history of HNPGL/PPGL, multiple tumors, extra-adrenal location, or early age at diagnosis (mean age at diagnosis in SDHB/SDHD-related tumors is ~10 years earlier than in sporadic cases) can be used to stratify the likelihood of a germline mutation being detected and so increase cost-effectiveness by targeting higher risk subgroups (Erlic et al. 2009). The application of such testing protocols was assessed in an audit of a referral-based testing series of SDHB, SDHD, and VHL (Jafri et al. 2013), and it was demonstrated that though widening the testing criteria for testing sporadic pheochromocytoma cases (e.g. from only those aged < 45 years at diagnosis to those aged < 60 years) increased the numbers of mutation carriers tested but the cost of detecting each mutation carrier increased. A complementary approach is to undertake immunohistochemical analysis for SDHB protein expression in the tumors of patients who fall outside the selection criteria. Though loss of SDHB expression is a sensitive and specific indicator of germline SDHX mutations (van Nederveen et al. 2009), tumor material may not always be available to evaluate protein loss. However, as additional inherited HNPGL/PPGL genes have been identified, there has been increasing interest in the application of next generation sequencing strategies to allow comprehensive and less expensive genetic analysis. Thus specific targeted resequencing panels and exome analysis strategies have been described (Rattenberry et al. 2013, McInerney-Leo et al. 2014). As the cost of genetic analysis falls, it seems likely that there will be a move towards more extensive analysis.

**Pathogenesis of SDH-mutated PPGLs**

Pathogenesis of PPGLs caused by SDH mutations remains poorly understood. SDH catalyzes the oxidation of succinate to fumarate in the Krebs cycle and functions as mitochondrial complex II by transferring the extracted electrons to ubiquinone in the electron transport chain. Loss of SDH activity leads to increased succinate and reactive oxygen species (ROS). Thus, succinate and ROS are considered as the signaling molecules that ultimately trigger tumor formation upon SDH mutations. Since discovery of the first mutations in familial PPGLs in 2000–2001 (Baysal et al. 2000, Niemann & Muller 2000, Astuti et al. 2001b), alternative models for tumor development have been advanced using different observations and experimental models that studied the consequences of SDH genetic loss. These models can be broadly classified as constitutive hypoxic drive, inhibition of developmental neuronal culling and histone/genome hypermethylation (Fig. 3).

**Constitutive hypoxic drive**

The most common phenotypic manifestation of germline SDH mutations is the development of PPGL tumors
Gastrointestinal stromal tumors (Janeway et al. 2011, Pantaleo et al. 2011) and renal carcinoma (Neumann et al. 2004, Ricketts et al. 2008) also develop in a small minority of subjects who carry germ line SDH mutations. HNPGL, especially the carotid body (CB) paraganglioma, are characteristically associated with germ line mutations in structural subunit genes SDHD, SDHC, SDHB, and in regulatory subunit genes SDHAF2 (Boedeker et al. 2014). The CB is an acute oxygen-sensing organ that responds to hypoxia by increasing heart and ventilation rate (Lopez-Barneo et al. 2008). It has been recognized that the incidence of CB paragangliomas increase among high altitude dwellers (Saldana et al. 1973) and those with chronic cyanotic heart diseases (Lack 1978, Opotowsky et al. 2015). These observations suggested early on that the SDH mutations disrupt oxygen sensing of the CB by causing an inability to register presence of normal oxygen levels (Baysal et al. 2000). The paraganglioma tumor formation may thus follow chronic hypoxic stimulation of the CB oxygen-sensing (chief) cells, either by environmental hypoxia or by SDH mutations that inhibit oxygen sensing. The hypothesis that chronic (pseudo)hypoxic stimulation may lead to hereditary paragangliomas is also supported by evidence that links increased altitudes to increased severity of SDH-mutated paraganglioma tumors (Astrom et al. 2003, Cerecer-Gil et al. 2010).

Recent genome-wide expression profiling studies show strong induction of hypoxia and angiogenesis pathways in SDH- and VHL-related PPGL (Dahia et al. 2005, Lopez-Jimenez et al. 2010, Shankavaram et al. 2013). SDH and VHL mutations induce both protein encoding mRNAs and miRNAs (miR-210; Tsang et al. 2014) that are implicated in cellular adaptation to hypoxia. Although certain differences in the induced genes were observed, the broad overlap amongst the hypoxia induced genes between SDH- and VHL-related paragangliomas strongly suggest that pathogenesis of SDH tumors involves constitutive hypoxic stimulation.

The VHL gene product (pVHL) is a component of the protein complex that possesses ubiquitin ligase activity which mediates the proteosomal degradation of hypoxia-inducible factors (HIFs) under normoxia (Gossage et al. 2015). HIFs (HIF1α, HIF2α, and HIF3α) are transcription factors that mediate cellular adaptation to hypoxia (Semenza 2012). HIFα subunits are hydroxylated by prolyl or asparaginyl hydroxylase enzymes (PhD1, PhD2, PhD3, and FIH) in normoxia and subsequently degraded by ubiquitination (Kaelin & Ratcliffe 2008). Hypoxia inhibits the hydroxylase enzymes and leads to stabilization of HIFαs. It is thought that mutations in VHL lead to tumor formation through constitutive stabilization of HIFs.
Role of HIFs in SDH-mutated PPGLs

Broad transcriptional overlap between SDH and VHL-related PPGL, and constitutive activation of the HIFs in VHL suggested that HIFs may also mediate tumor formation in SDH-mutated paragangliomas. HIF1α and/or HIF2α were detected by immunohistochemistry in both SDH-mutated and sporadic HNPGL (Pollard et al. 2006, Favier et al. 2009, Merlo et al. 2012). In vitro studies using cell lines showed that siRNA-mediated knockdown of SDH subunits led to stabilization of HIF1α (Selak et al. 2005, Cervera et al. 2008, Guzy et al. 2008). These studies linked increased succinate or ROS levels to the stabilization of HIFαs. Despite these in vitro studies, discordant results are obtained from gene expression analyses on the role of HIFs in SDH-mutated PGL tumors. Although significant overlap in gene expression patterns of SDH- and VHL-related PGL tumors was observed including increased HIF2α, VEGF and reduced electron transport chain genes by transcriptome-wide studies, HIF target-gene overexpression and increased glycolysis, as assessed by such genes as hexokinase II (HK2), lactate dehydrogenase, MIR210, PHD3 (EGLN3), ENO1, and SLC2A1 were primarily observed in VHL-mutated paragangliomas (Favier et al. 2009, Lopez-Jimenez et al. 2010). In fact, overexpression of 67 HIF target genes was sufficient to distinguish VHL- from SDHB-mutated pheochromocytomas (Lopez-Jimenez et al. 2010). Conversely, HNPGLs that overexpress HIF1α and its target genes were found to have WT SDH sequences and a subset of them was indeed found to carry somatic VHL mutations (Merlo et al. 2012, 2013).

In addition, recent sequence and functional studies identified somatic mutations in EPAS1 which encodes the HIF2α subunit in sporadic (mostly non-head and neck) paragangliomas, a subset of which was accompanied by polycythemia (Zhuang et al. 2012, Comino-Mendez et al. 2013, Toledo et al. 2013). Missense mutations in VHL, EPAS1 (HIF2A), and PHD2 are associated with erythrocytosis. For example, endemic Chuvash polycythemia is caused by certain VHL missense germ line mutations (Lee & Percy 2011). In contrast, SDHX mutations have yet to be associated with erythrocytosis. Gene expression profiling shows that EPAS1-mutated paragangliomas cluster with SDH and VHL-related paragangliomas, and strengthens the role of constitutive hypoxic signaling in pathogenesis of SDH-mutated paragangliomas (Comino-Mendez et al. 2013). However, the association with erythrocytosis suggests that pathogenesis of EPAS1-mutated paragangliomas is more closely associated with the VHL-mutated paragangliomas rather than with the SDH-mutated ones.

It thus appears that gene expression studies do not provide an unequivocal evidence for involvement of HIFs in pathogenesis of SDH-mutated paragangliomas. Whether HIFs play a role in SDH-paraganglioma formation, however, remains an important question which may not be conclusively answered by gene/protein expression studies alone. It is important to note that hereditary renal tumors that result from fumarate hydratase (FH) germline mutations were initially shown to stabilize HIF1α (Pollard et al. 2005). Accordingly, the knockdown of FH in cell lines led to robust stabilization of HIF1α through fumarate mediated inhibition of the PhD enzymes (Isaacs et al. 2005). These findings are similar to the observations previously described for SDH-mutated pathology: i) HIFαs are variably detected in SDH-mutated paragangliomas by gene expression and immunohistochemical studies and ii) succinate inhibition of PhD enzymes stabilizes HIF1α upon SDH knockdown in certain cell lines. Although such observations initially suggested a role for HIF1 in tumor predisposition caused by FH mutations, deletion of the Hif1α gene in the Fh1-deficient mice, which develops renal cysts, worsened the cystic phenotype (Adam et al. 2011). Thus, Hif1α may not mediate the cystic renal pathology in the Fh1-mice. These results imply that HIF stabilization observed in SDH-mutated tumors may not necessarily indicate its causative role in tumor pathogenesis. Ultimately, animal or cell culture models that link inactivation of SDH to PPGL development or to a hypoxia-related physiological response will be required to evaluate the role of HIFs in SDH-mutated tumor pathogenesis or SDH-regulated hypoxia response.

Heterozygous inactivation of Sdhd or Sdhd genes in mice does not lead to tumor development, in particular while homozygous inactivation is embryonic lethal (Bayley et al. 2009a,b, Piruat & Millán-Ucled 2014). Sdhd conditional constitutional or paraganglia-confined homozygous deletions also show no evidence of tumor development (Díaz-Castro et al. 2012). These findings highlight species-specific differences in tumor susceptibility between human and mouse that follows the inactivation of mitochondrial complex II subunits. While gene knockout studies in mice did not recapitulate the paraganglioma tumor phenotype, they provide some information on activation of hypoxia-related pathways. Heterozygous Sdhd deletion increases sensitivity of the CB chief cells to hypoxia (Piruat et al. 2004), which is consistent with the hypothesis that inactivation of Sdh hampers the ability of CB chief cells to register normal oxygen levels and triggers normoxic activation of hypoxia sensing pathways. Gene expression analyses of Sdhd−/− tissues show mixed evidence of Hif activation. While Sdhd−/− MEFs showed Hif1α...
stabilization, two other tissues did not show any evidence of hypoxic pathway activation (Millán-Ucles et al. 2014).

In summary, mimicry of the hypoxia-associated CB paragangliomas and gene expression profiling studies provide strong evidence of constitutive hypoxic pathway activation in pathogenesis SDH-mutated paraganglioma tumors. However, determining the role of HIFs in mediating this hypoxia-driven pathogenesis requires further studies.

**Inhibition of α-ketoglutarate dependent dioxygenases**

The PhD enzymes are members of a large family of Fe(II)/α-ketoglutarate (KG)-dependent dioxygenases (Hausinger 2004). Inhibition of PhDs by succinate on genetic and pharmacologic inhibition of SDH raised the possibility that other dioxygenases may also contribute to paraganglioma development. Succinate accumulation by SDH inhibition has been shown to inhibit jumonji-domain histone demethylases (JmC), leading to histone H3 hypermethylation (Smith et al. 2007). Succinate is also shown to inhibit other α-KG-dependent dioxygenases, including collagen prolyl-4-hydroxylases and the ten-eleven translocation (TET) family of 5-methylcytosine (5mC) hydroxylases, which leads to hypermethylation of CpG islands (Xiao et al. 2012). Both histone and DNA hypermethylation have the potential to alter gene expression levels. Examination of SDH-mutated paragangliomas showed downregulation of gene expression for 191 genes that acquired promoter methylation as a result of inhibition of the TET family of 5mC hydroxylases (Letouzé et al. 2013). Certain methylated genes including PNMT and KRT19 were linked to neuroendocrine differentiation and epithelial-to-mesenchymal differentiation, respectively, raising the possibility that succinate-mediated inhibition of TET family 5mC hydroxylases may play a role in SDH-mutated paraganglioma development. Whether suppression of PNMT, KRT19, or other genes by CpG island or histone methylation provides an advantage in SDH-mutated tumor progression, however, remains to be directly demonstrated. As previously discussed, broad overlap in hypoxia-related gene expression patterns between SDH and VHL paragangliomas and between genetic and sporadic HNPLG suggests that the role of histone and DNA methylation in influencing global gene expression profiles may be limited. It is conceivable that whereas initiation of SDH-mutated paragangliomas may involve constitutive hypoxia-signaling, succinate-inhibition of α-KG-dependent dioxygenases may contribute to tumor progression.

Germ line mutations in FH in hereditary leiomyomatosis and renal cell cancer, somatic gain-of-function point mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 in low-grade gliomas, secondary glioblastomas, various sarcomas, and acute myeloid leukemia cause increased fumarate and α-2-hydroxylutarate, respectively, and lead to DNA and histone methylation by inhibiting α-KG-dependent dioxygenases (Morin et al. 2014). α-KG-dependent dioxygenases comprise a large family of enzymes that perform diverse and important biological functions including protein modification, repair of alkylated DNA/RNA, and lipid metabolism. It is notable that there is no major overlap amongst the tumor spectra associated with SDH, FH, and IDH1/2 mutations, although rare familial paraganglioma cases carrying FH germ line mutations have been recently described (Letouzé et al. 2013, Clark et al. 2014). Thus, succinate, fumarate, and α-2-hydroxylutarate, the oncometabolites generated by SDH, FH, and IDH1/2 mutations, respectively, may inhibit not only PHD, TET, and histone demethylating enzymes but also other α-KG-dependent dioxygenases that provide an advantage for cancer cell survival.

Whether succinate receptors (SUCNR) mediate signaling in pathogenesis of SDH-mutated paragangliomas remains an underexplored area. Succinate is a ligand for GPR91 (also known as SUCNR1), a G-protein coupled receptor (He et al. 2004). SUCNR1 is expressed in kidney, liver, spleen and white adipose tissue (Ariza et al. 2012). SUCNR1 stimulates angiogenesis in retina. Extracellular succinate activates the SUCNR and may increase the VEGF levels through HIFα-independent mechanisms (Sapienza et al. 2008). SUCNR1 may mediate certain effects of hypoxia which increases the succinate levels. These results suggest that succinate can act as a physiological signaling molecule and raise the possibility that some aspects of paragangliomas, such as increased vascularity may be mediated by increased succinate signaling through SUCNR1.

**Inhibition of neuronal apoptosis linked to PHD3**

It has been suggested that succinate accumulation following SDH inactivation inhibits PHD3 activity, which is required for neuronal apoptosis after NGF withdrawal (Lee et al. 2005). According to this model, abnormal NGF signaling leading to reduced apoptosis and enhanced survival of sympathetic neurons provides a unifying model for the mechanism of pheochromocytoma formation following mutations in NFI, RET, SDH, and VHL. Notably, Lee et al. (2005) did not observe HIF stabilization in PC12 pheochromocytoma cell lines after SDH knockdown and suggested that succinate inhibition of PHD3, rather PHD1 which controls HIF stability,
provides a mechanistic link between SDH inactivation and pheochromocytoma susceptibility (Lee et al. 2005). It is conceivable that inhibition of neuronal apoptosis and epigenetic inactivation of genes important for neuronal differentiation by TET inactivation may collaborate to promote development of paraganglioma tumors. However, it is unclear whether this common model based on inhibition of apoptosis can explain the distinct expression profiles conferred by mutations in VHL/SDHX vs RET/NFI in PPGLs and the activation of hypoxia-related pathways specifically in the SDH-mutated paragangliomas.

Other aspects of pathogenesis in SDH-mutated paragangliomas

Role of ROS in SDH-mutated pathogenesis

Mitochondrial complex II generates significant quantities of ROS (Quinlan et al. 2012), which is further enhanced by certain mutations (Ishii et al. 2005). Whether ROS contributes to pathogenesis of SDH-mutated paragangliomas is the subject of ongoing investigations. In addition to stabilizing HIF1α (Guzy et al. 2008), ROS generated by SDHC mutations has also been implicated in mutating nuclear DNA and therefore contributing to tumorigenesis (Ishii et al. 2005). Role of somatic mutations in SDH-mutated paragangliomas, however, remains unconfirmed. SDH-mutated paragangliomas does not frequently acquire point mutations in the non-mutated allele, which is often lost by large deletions (Dahia 2014). Recent tumor sequencing studies also show very low levels of overall mutations in SDH-mutated paragangliomas (Castro-Vega et al. 2015).

Malignancy among SDHB mutation carriers

Prevalence of malignant paragangliomas as defined by metastasis among SDHB mutation carriers is substantially higher than among SDHD carriers (13% vs 4%; van Hulsteijn et al. 2012). The association of SDHB mutations with malignancy appears to hold both for HNPGL and non-HNPGL (Boedeker et al. 2007). Metastasis is thought to occur through a process called epithelial–mesenchymal transition (EMT; Scheel & Weinberg 2012). EMT confers cancer cells with stem-cell like properties including the ability to migrate and grow in distant anatomic sites. Gene expression analyses of SDHB-related metastatic paragangliomas show differential alterations in genes implicated in EMT, such as those encoding metalloproteinases and cellular junction proteins (Loriot et al. 2012). Whole exome-sequencing identified ATRX2 mutations in subset of clinically aggressive paragangliomas, including in two SDHB-mutated tumors (Fishbein et al. 2015). While such findings may help to explain the mechanism by which the metastatic behavior is acquired in SDHB–paragangliomas, the question remains as to why the loss of SDH through different subunit gene mutations have such distinct consequences on the metastatic potential.

Because heterozygous SDH mutations predispose to tumor formation, haploinsufficiency of an SDH subunit initiates the tumorigenic process. Hereditary paraganglioma formation usually follows loss of the unmutated SDHB or SDHD allele, which abolishes the whole mitochondrial complex II activity. It is conceivable that SDHB haploinsufficiency occurs in developmentally more immature paranganglionic cells that are prone to develop stem-cell like properties during tumorigenesis enabling them to migrate and proliferate in distant sites. However, SDHD or SDHC haploinsufficiency may occur in more mature paranganglionic cells that are less likely to dedifferentiate and metastasize.

Conclusion

Mutations in SDH subunits account for most familial and sporadic HNPGLs and PGGLs, and have also been linked to other neoplasms including GISTs, renal cancer, and pituitary adenomas. Abundant evidence suggests that constitutive hypoxic stimulation plays an important role in development of SDH-mutated paraganglioma tumors. However, mechanisms by which SDH regulates oxygen sensing and signaling are poorly understood. Progress would be facilitated by development of relevant animal or cell culture models that link SDH dysfunction to tumor formation and/or to altered physiological responses to hypoxia. Whether HIF1α/HIF2α is involved in the hypoxic signaling pathway suspected to drive SDH-mutated paragangliomas can be rigorously addressed only through such models. Recent studies on Fh1 mouse model suggest that the stabilization of HIFs in tumor samples does not necessarily indicate its involvement in tumor pathogenesis. Although the association of activating EPAS1/HIF2A mutations with PGGL would support a role for HIFs, the co-occurrence of erythrocytosis in certain carriers suggests that pathogenesis of PGGL tumors with EPAS1/HIF2A mutations may be more closely associated with VHL-mutated than SDHX-mutated tumors. Succinate accumulation in SDH-mutated paragangliomas inhibits certain α-KG-independent dioxygenases and leads to histone
and DNA hypermethylation. Significance of these hypermethylation events in driving PPGL tumor formation and whether they influence hypoxic pathway activation requires further studies. The discovery of SDH mutations in PPGLs in early the 2000s confirmed Warburg’s suspicion that defective mitochondria is the root cause of the neoplastic process (Warburg 1956) at least in certain tumor types, and heralded an era of metabolic studies that aim to understand the role of mitochondria in cancer (Wallace 2012).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received in final form 24 June 2015
Accepted 25 June 2015
Made available online as an Accepted Preprint
25 June 2015