Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity

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Abstract | The neuroendocrine tumours pheochromocytomas and paragangliomas carry the highest degree of heritability in human neoplasms, enabling genetic alterations to be traced to clinical phenotypes through their transmission in families. Mutations in more than a dozen distinct susceptibility genes have implicated multiple pathways in these tumours, offering insights into kinase downstream signalling interactions and hypoxia regulation, and uncovering links between metabolism, epigenetic remodelling and cell growth. These advances extend to co-occurring tumours, including renal, thyroid and gastrointestinal malignancies. Hereditary pheochromocytomas and paragangliomas are powerful models for recognizing cancer driver events, which can be harnessed for diagnostic purposes and for guiding the future development of targeted therapies.

Catecholamine
Hormone produced by the chromaffin cells of the adrenal medulla and the postganglionic fibres of the sympathetic nervous system; the main catecholamines are noradrenaline, adrenaline and dopamine.

Hypoxia
Reduced oxygen content, below physiological levels.

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doi:10.1038/nrc3648
Published online
20 January 2014

Pheochromocytomas and paragangliomas are highly vascular, catecholamine-secreting tumours that arise from sympathetic lineage-derived cells from the adrenal medulla (pheochromocytomas) and from extra-adrenal thoracic and abdominal paraganglia (paragangliomas)[1–3]. These tumours differ from paragangliomas of parasympathetic origin, which arise in the head and neck and are usually unable to secrete catecholamines[3]. Pheochromocytomas and paragangliomas, sometimes referred to collectively as paraganglial tumours, are predominantly benign. However, approximately 10–15% can develop metastases to embryologically unrelated tissue, including bone, liver, lungs and lymph nodes[4]. Malignant pheochromocytomas and paragangliomas remain a diagnostic and therapeutic challenge owing to limited knowledge of markers of malignancy and a lack of effective treatment options[4].

Approximately 40% of pheochromocytomas and paragangliomas carry a germline mutation in one of at least 12 genes[5,6] (FIG. 1). These susceptibility genes belong to a wide range of functional classes, including kinase receptor and signalling regulators (such as *RET* and neurofibromin 1 (*NF1*)); transcription factors (such as MYC-associated factor X (*MAX*)); energy metabolism components (such as succinate dehydrogenase (SDH) subunits *SDHA*, *SDHB*, *SDHC*, *SDHD* and cofactor *SDHAF2*); constituents of the cellular response to hypoxia (such as von Hippel–Lindau (*VHL*) and hypoxia-inducible factor 2A (*HIF2A*; also known as *EPAS1*); and endosomal signalling (such as transmembrane protein 127 (*TMEM127*)). Furthermore, somatic mutations of *RET*, *VHL*, *NF1*, *MAX* and *HIF2A* can be detected in an additional 25–30% of the tumours[7–10] (FIG. 1). Mutations, both hereditary and somatic, are found in a mutually exclusive manner in these tumours, which points to the redundancy of the affected signals. The convergence of disrupted pathways is further supported by the transcription distribution of these tumours, in which genetic lesions share common signalling aberrations[11]. In aggregate, the combined genomic and genetic efforts of the past decade have uncovered a genetic driver event in more than 50% of pheochromocytomas and paragangliomas[12].

These neoplasms have been paradigm shifters: they were the first human tumour model found to carry an inherited mutation of a gene encoding a metabolic enzyme, *SDHD*[13]. They are also pioneer models of genetic-based personalized medical care as they are components of multiple endocrine neoplasia type 2 (MEN2) syndrome, in which the identification of a high-risk germline *RET* mutation can guide the need for early thyroidectomy to prevent medullary thyroid carcinoma, which is a co-occurring tumour in this disorder[14–16]. More recently, they also became the first tumours known to carry activating mutations of *HIF2A*, which had long been implicated in multiple human cancers and was suspected, but had never been genetically proved, to function as a bona fide oncogene[10,17–20].

Key points

- Pheochromocytomas and paragangliomas carry the highest degree of heritability (around 40%) of all human tumours and thus represent relevant models for the identification of driver mutations in cancer.
- Genetic testing of inherited mutations allows the identification of co-occurring cancers in hereditary syndromes and screening of at-risk relatives, with an impact on health care.
- More than 12 genes, belonging to a wide range of functional classes are mutated in the germ line or, less frequently, in somatic pheochromocytomas and paragangliomas, but many tumours remain genetically undefined.
- Two main transcription signatures, associated with hypoxia-related signals (cluster 1) and increased kinase signalling (cluster 2), underlie the various driver mutations, revealing pathway interactions and enabling the discovery of novel predisposing genes.
- Mutations of metabolism genes uncovered the cell growth-promoting effects of metabolism intermediates (succinate) through epigenetic (histone and DNA methylation) modulation and activation of a hypoxic response.
- Mechanisms involved in the malignant transformation of pheochromocytomas and paragangliomas are not fully elucidated, and treatment options for these tumours are still limited.

Despite being predominantly benign tumours, pheochromocytomas and paragangliomas have substantially advanced our understanding of cancer biology. This Review addresses the genetic and molecular effects of the various reported mutations, with an emphasis on the constitutive activation of hypoxic pathways owing to increased stability of HIFs (a phenomenon also known as pseudohypoxia), and epigenetic changes that occur in some of these models as a result of metabolite imbalances. Activation of common and unique downstream signals involving RAS, PI3K–AKT and mTOR by mutations of *RET*, *NF1*, *TMEM127* and *MAX* are also reviewed. Finally, areas of active investigation are briefly discussed, including ongoing genomic studies to uncover the molecular basis of the remaining 'orphan' pheochromocytomas and paragangliomas, identification of actionable targets of malignancy and recurrence, and the increased risk of concurrent cancers as part of inherited pheochromocytoma and paraganglioma syndromes.

Molecular pathogenesis

Using the information from inherited syndromes in which patients develop pheochromocytomas and paragangliomas, detailed clinical and genetic assessments of patients and their families have defined genotype–phenotype associations that inform risk stratification, patient surveillance and screening of at-risk relatives, all of which have had an impact on health care. These themes have been extensively reported[3,5,21,22] and are not discussed in this Review. TABLE 1 summarizes the main distinguishing features of pheochromocytomas and paragangliomas that are associated with each recognized susceptibility gene. With the rapid expansion of routine screening strategies to include new susceptibility targets and the adoption of improved methodology that captures a broader range of gene disruptions, the actual population frequency of mutation-positive cases is now being better defined. These numbers suggest that a germline mutation is recognized in almost 50% of patients with features of inheritability (such as early age at onset, multiple tumours and/or family history of pheochromocytoma, paraganglioma or other syndrome-associated tumours), whereas causal mutations have been identified in only a small subset (10%) of patients who present with typical sporadic disease[23]. Recently, somatic driver mutations have begun to be recognized, and these findings account for an increasingly larger proportion of the sporadic cases (as discussed below).

Pheochromocytomas and paragangliomas of various genetic backgrounds can be segregated by their transcription profile into two main clusters (cluster 1 and cluster 2) that have helped to guide the discovery of novel susceptibility genes. Cluster 1 is enriched for genes that are associated with the hypoxic response, and cluster 2 contains tumours that activate kinase signalling and protein translation[11,12,24,25]. Cluster 1 contains tumours with mutations of *VHL*, components of the SDH complex (*SDHA*, *SDHB*, *SDHC* and *SDHD*, as well as *SDHAF2*) and *HIF2A*. Cluster 2, a more heterogeneous group, encompasses pheochromocytomas and paragangliomas with *RET*, *NF1*, *TMEM127* and *MAX* mutations.

Cluster 1: pseudohypoxia-driven tumours

A common feature of cluster 1 tumours is the activation of HIFs. These transcription factors are physiologically induced in response to low cellular oxygen levels (hypoxia)[26]. Pseudohypoxia occurs when HIF pathways are constitutively activated, regardless of oxygen levels[27]. HIFs are heterodimeric transcription factors and their inducible components, or α-subunits (HIF1α and HIF2α), are tightly regulated by hydroxylation and proteosomal degradation[27–31]. These processes are partly controlled by cluster 1 pheochromocytoma and paraganglioma genes (detailed below).

***VHL*-associated pseudohypoxia.** *VHL* mutations occur in the setting of von Hippel–Lindau disease, in which pheochromocytomas and paragangliomas associate with renal cell carcinomas of the clear cell histological type and with haemangioblastomas of the cerebellum, spinal cord and retina[30], but somatic *VHL* mutations are also detected in these tumours[12]. Although renal carcinoma-related mutations involve deletions and truncations that severely destabilize VHL function, pheochromocytoma- and paraganglioma-associated mutations are predominantly missense in nature[32].

VHL is the substrate recognition component of an E3 ubiquitin ligase complex, which targets substrates HIF1α and HIF2α for proteasome-mediated degradation[33] (FIG. 2). Thus, in *VHL*-mutant tumours, degradation of HIF1α and HIF2α is reduced, leading to HIF accumulation and subsequent induction of multiple downstream targets. Many HIF targets are thought to have a role in the transformed phenotype of the target tissues, and metabolic changes are a hallmark of HIF activation (BOX 1). Although many targets are shared between HIF1α and HIF2α, others are preferentially, or exclusively, activated by one or the other transcription factor (BOX 1), and this has led to the idea that HIF2α has more oncogenic properties[34,35]. Indeed, HIF2α promotes

Pseudohypoxia
Aberrant activation of hypoxia-inducible factor and induction of its target genes, regardless of oxygen levels.

Dioxygenases
Enzymes that require oxygen and the metabolite α-ketoglutarate as co-substrates. Important members of this class are hypoxia-inducible factor prolyl hydroxylases, Jumonji histone demethylases and TET DNA hydroxylases.

Jumonji (JMJ) demethylase
Dioxygenases of the histone lysine demethylase family, which remove methyl groups of lysines in histones that control active or silent gene expression.

TET
Methyl cytosine dioxygenases that hydroxylate 5-methylcytosine (5mC) to generate 5-hydroxymethylcytosine (5hmC); 5hmC produces G:C base-pair mismatches that are removed by thymine–DNA glycosylase, which results in broad DNA demethylation.

the activity of the oncogene MYC[36], whereas HIF1α can antagonize MYC[37]. In VHL-null renal carcinoma, HIF2α was shown to be both necessary[38] and sufficient[39] for tumour formation in mice, in further support of an oncogenic role for this protein. Moreover, most *VHL* mutations preferentially impair degradation of the HIF2α subunit compared with HIF1α *in vitro*[40]. Consistent with this finding, HIF2α is preferentially upregulated in *VHL*-mutant pheochromocytomas and paragangliomas[24,41,42], and activating *HIF2A* gene mutations have recently been found in pheochromocytomas and paragangliomas (discussed below)[10,17,18,20]. By contrast, HIF1α activation predominated in VHL-mutated pheochromocytomas and paragangliomas in at least one study[25]. The role of HIF1α in cancer is controversial: HIF1α has been considered, under some circumstances, to be a tumour suppressor, and deletions of the *HIF1A* locus have been detected in renal cell carcinomas[43], although no mutations or deletions spanning the *HIF1A* locus have been reported in pheochromocytomas and paragangliomas[10]. Thus, whether HIF1α functions as a tumour promoter or a tumour suppressor in VHL-mutant cells may be context or tissue dependent[35], or may involve epigenetic modulation[44], a concept that has recently been explored in the SDH model of pheochromocytomas and paragangliomas (discussed below).

Despite the clear relevance of HIF to VHL-mediated pathogenesis, mutant VHL can be tumorigenic independently of HIF activation[45,46]. HIF-unrelated *VHL* mutations were initially identified in patients with pheochromocytomas but who lacked other manifestations of von Hippel–Lindau disease, a syndrome that is known as VHL type 2C[30,47]. Some properties of these variants are shared by other pheochromocytoma- and paraganglioma-predisposing mutations (BOX 2). Additional HIF-independent functions of VHL have been extensively discussed elsewhere[47–49].

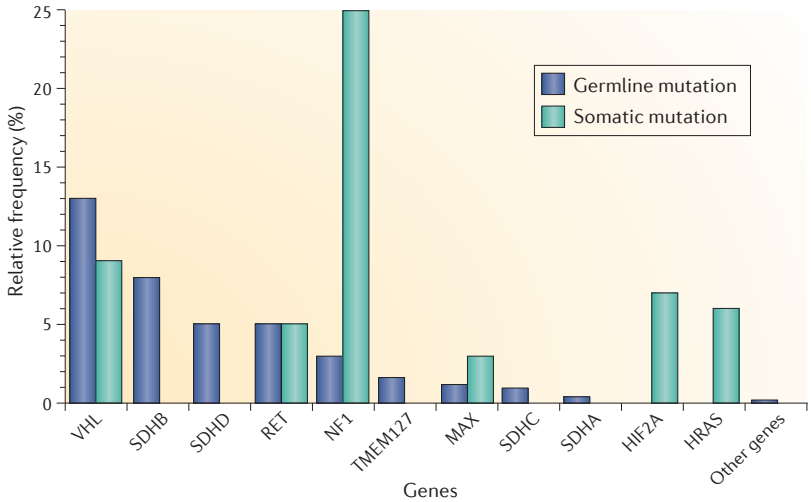

Figure 1 | **Relative frequencies of gene mutations in germline or somatic DNA of pheochromocytomas and paragangliomas.** Germline (shown in blue) and somatic (green) mutation frequencies in indicated genes are shown. The 'Other genes' category refers to those that were reported in single families and/or cases including *KIF1B*, prolyl hydroxylase domain-contaning protein 2 (*PHD2*) and fumarate hydratase (*FH*). Data were obtained from multiple published series, and frequencies may reflect ascertainment biases. *HIF2A*, hypoxia-inducible factor 2A; MAX, MYC-associated factor X; *NF1*, neurofibromin 1; *SDH*, succinate dehydrogenase; *TMEM127*, transmembrane protein 127; *VHL*, von Hippel–Lindau.

SDH-related pseudohypoxia. Other pseudohypoxic pheochromocytomas and paragangliomas are those caused by mutations in SDH-component enzymes, and this is arguably the area that has experienced the most substantive advances in the field[11,12]. SDH is a heterooligomer that contains the subunits SDHA, SDHB, SDHC and SDHD[50]. In addition, at least two other proteins, SDHAF1 and SDHAF2, contribute to the functional SDH by flavinating the SDHA subunit and thus enabling the assembly of the full SDH complex[50]. The two main functions of SDH are the oxidative dehydrogenation of succinate to fumarate in the tricarboxylic acid cycle (TCA) cycle (FIG. 2) and the reduction of ubiquinone in the electron transport chain during ATP synthesis[50].

Germline loss-of-function mutations of all four SDH subunits and *SDHAF2* have been linked to unique hereditary paraganglioma and/or pheochromocytoma syndromes, which are known as PGL1–4 (REFS 13,51–54) and, less frequently, to renal cell carcinomas and gastrointestinal stromal tumours (GISTs), and more recently to pituitary adenomas[55–57] (TABLE 1). *In vivo* and *in vitro* evidence has highlighted the relevance of succinate, which accumulates as a result of the loss of SDH activity, as a key element in the pathogenesis of these tumours.

Succinate, the substrate of the SDH reaction, affects HIF stability through its effects on post-translational regulation of HIFα subunits, an essential step for the recognition of HIF for proteasome-mediated degradation. Stability of HIFα subunits is dependent on their hydroxylation at specific proline residues by a class of dioxygenases, the prolyl hydroxylases (PHDs) type 1 (PHD1; also known as EGLN2), PHD2 (also known as EGLN1) and PHD3 (also known as EGLN3)[28,29]. The activity of HIF PHDs is regulated by oxygen, iron, ascorbate and α-ketoglutarate (αKG; also known as 2-oxoglutarate), which is converted by PHDs into succinate. In SDH deficiency succinate accumulates and, because of its structural similarity to αKG, competitively inhibits the activity of PHDs in the cytosol[58]. In agreement with these findings, pheochromocytomas and paragangliomas with SDH mutations have increased stability of HIF and increased expression of HIF targets[11,12,25].

Accordingly, *SDHA* or *SDHB* knockdown not only phenocopied succinate-dependent inhibition of PHDs, but also led to the inhibition of other classes of αKG-dependent enzymes: histone demethylases of the Jumonji (JMJ) demethylase family and TET hydroxylases[59]. Inhibition of these dioxygenases by SDH downregulation led to the induction of HIF target genes, as well as to DNA and histone hypermethylation. These changes were all reversible by αKG supplementation or re-expression of wild-type but not of pheochromocytoma- and paraganglioma-related mutant versions of the *SDHA* or *SDHB* genes[59]. Aligned with these results in cell lines, SDH-mutant pheochromocytomas and paragangliomas were recently shown to display a global pattern of hypermethylation that was accompanied

Table 1 | **Summary of common clinical presentations of pheochromocytomas and paragangliomas with detectable mutations**

Genes	Predominant tumour site	Tumour number (multiple versus single)	Family history (relative frequency)	Malignancy risk	Related conditions	Refs
NF1	Pheochromocytoma > paraganglioma	Single	High	Moderate	Neurofibromas, MPNSTs and gliomas	7,9
RET	Pheochromocytoma	Multiple	High	Low	MTC, hyperparathyroidism and marfanoid habitus	3,5–7,12,22
VHL	Pheochromocytoma > paraganglioma	Multiple	High	Low	RCCs and CNS hemangioblastomas	3,5–7,12,22
SDHA	Paraganglioma	Single	Low	?*	GISTs	3,5,7,12,23,56
SDHB	Paraganglioma > pheochromocytoma	Multiple	Low	High	GISTs and RCCs	3,5,7,12, 23,55–57
SDHC	Paraganglioma	Multiple	Low	Low	GISTs	3,5,7,12,23,56
SDHD	Paraganglioma > pheochromocytoma	Multiple	High	Low	GISTs and pituitary adenomas	3,5,7,12, 23,56,57
SDHAF2	Paraganglioma	Multiple	High	?	None reported	3,5,54
TMEM127	Pheochromocytoma	Single	Moderate to low	Low	None reported.* RCC recently described, although not in association with pheochromocytoma.	111,123, 124,125
MAX	Pheochromocytoma > paraganglioma	Single	Moderate to low	Low	None reported	8,112
HIF2	Paraganglioma > pheochromocytoma	Multiple	?	?	Polycythemia and somatostatinomas	10,17,18, 20,80
KIF1B	Pheochromocytoma?	?	?	?	Neuroblastoma?	150,151
PHD2	Paraganglioma?	?	?	?	Polycythemia	81
HRAS	Pheochromocytoma?	Single	?	?	None reported; gene mutated in multiple cancers‡	135
FH	Pheochromocytoma?	?	?	?	Uterine leiomyoma	60

CNS, central nervous system; *FH*, fumarate hydratase; GISTs, gastrointestinal stromal tumours; *HIF2*, hypoxia-inducible factor 2; *MAX*, MYC-associated factor X; MPNSTs, malignant peripheral nerve sheath tumours; MTC, medullary thyroid carcinoma; *NF1*, neurofibromin 1; *PHD2*, prolyl hydroxylase domain-containing protein 2; RCC, renal cell carcinoma; *SDH*, succinate dehydrogenase; *TMEM127*, transmembrane protein 127; *VHL*, von Hippel–lindau. *Represents unknown or insufficient data. ‡*HRAS* mutations are detected in multiple human cancers, including medullary thyroid carcinoma, bladder cancer, skin papillomas and salivary gland cancer.

by increased histone methylation and reduced expression of 5-hydroxymethylcytosine (5hmC), suggesting that in primary tumours with SDH mutations both DNA demethylation and histone demethylation are impaired[60]. Furthermore, in this study, chromaffin cells derived from an *Sdhb*-null mouse exhibited a methylation profile similar to that of SDH-mutant pheochromocytomas and paragangliomas, which could be corrected by the addition of a demethylase inhibitor, decitabine. This finding supports a role for SDH in regulating gene methylation *in vivo* and suggests that the effects of global hypermethylation can be potentially reversible therapeutically[60].

Interestingly, the epigenomic changes of SDH-mutant pheochromocytomas and paragangliomas were also described in a distinct type of SDH-deficient cancers, GISTs[61]. Furthermore, other cancer models with well-known hypermethylator phenotypes, including colorectal cancer (a specific subtype known as CpG island methylator phenotype (CIMP)) and glioblastomas carrying mutations in the metabolic enzymes encoded by isocitrate dehydrogenase 1 (*IDH1*) or *IDH2* (G-CIMP), overlap with the SDH-mutant methylator profile, lending additional support to the relevance of SDH in epigenetic modulation[60,61]. Although most of the effects of αKG and succinate imbalance of SDH deficiency have focused on impaired dioxygenase activity, these two metabolites have other functions, and it is currently unknown whether these properties contribute to the tumorigenic process in this model[62–65].

Deficiency of SDH has often been likened to loss of function of fumarate hydratase (FH), the enzyme that is mutated in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome[66]. This link was strengthened by the recent identification of *FH* gene mutations in a pheochromocytoma that displayed transcriptional and methylation similarities to SDH-mutant tumours[60]. FH catalyses the reaction that follows SDH in the TCA cycle and converts fumarate into malate (FIG. 2). Deficiency in FH activity results in the accumulation of the precursor metabolite, fumarate, which shares structural similarities with succinate[67] and, similarly, affects the same classes of αKG-dependent enzymes[59]. However, in FH deficiency, both HIFα accumulation and inhibition of JMJ histone demethylase are dependent on reactive oxygen species (ROS)[68], and ROS levels are increased in *FH*-mutant cells and tumours[69].

Chromaffin cells
Cells of neural crest origin thought to be the cell of origin of pheochromocytomas and sympathetic paragangliomas.

Somatostatinomas
Rare neuroendocrine tumours that produce the hormone somatostatin and arise from the pancreas, duodenum or bile ducts and can occur in isolation or associated with tumour syndromes such as neurofibromatosis type 1 or multiple endocrine neoplasia type 1.

Polycythemia
An abnormal increase in the number of circulating red cells. Occurs as a result of genetic defects (congenital) or in response to physiological or pathological conditions, including hypoxia.

By contrast, ROS detection in SDH-deficient cells has been controversial[58,70–73] and ROS levels were not increased in primary SDH-mutant pheochromocytomas and paragangliomas[74]. The parallels between SDH and FH models not only underscore the relevance of metabolic and epigenetic disruptions that lead to aberrant cellular growth, but also highlight the various mechanisms that underlie these changes.

***HIF2A*-associated pseudohypoxia.** As mentioned above, mutations in *HIF2A* were recently identified in pheochromocytomas and paragangliomas[10,17,18,20,75,76]. These mutations predominantly target one of the HIF2α-stabilizing prolyl sites, Pro531, or occur in proximity to this region. *In vitro* and structural studies have predicted that these mutations affect the conformation of HIF2α, which disrupts binding to PHDs[77] and VHL[78]. As expected, *in vitro*, *HIF2A* mutations led to a loss of recognition by PHDs, inability to bind to VHL and, consequently, to prolonged HIF2α half-life with induction of its downstream targets[10,17,18,20,76]. In addition, mutations targeting the 531 codon induced tumour growth *in vivo*, supporting a role for *HIF2A* as an oncogene in pheochromocytomas and paragangliomas[10]. Amplification of the mutant *HIF2A* allele was detected in some tumours, in agreement with a selective advantage conferred by the mutation in these cases[17,79]. Intriguingly, despite the somatic nature of the pheochromocytoma and paraganglioma mutations, a substantial proportion of patients also developed somatostatinomas that carried the same *HIF2A* variant[20,80]. In addition, approximately 50% of the patients with *HIF2A*-mutant tumours developed early onset or congenital polycythemia[17,76,80]. Germline *HIF2A* mutations have been reported in certain forms of polycythemia (familial erythrocytosis type 4), but no tumours have been previously described in these patients[81]. The apparent nonrandom association of pheochromocytomas and paragangliomas, somatostatinomas and early onset polycythemia suggests that these phenotypes might be linked (BOX 3). A germline *HIF2A* mutation was recently reported in a single patient with multiple paragangliomas and polycythemia but segregation of the mutation with the two clinical phenotypes could not be defined in this family[76], so it remains unclear whether germline transmission of *HIF2A* mutations can fully account for the syndromic phenotype.

HIF2A is required during the development of the sympathetic nervous system and chromaffin cells, potentially explaining why these tissues are uniquely vulnerable to tumour-causing mutations in this gene[82,83]. Interestingly, pheochromocytomas and paragangliomas with somatic *HIF2A* mutations showed increased transcription of genetic markers of less mature chromaffin cells, and genes involved in chromaffin cell differentiation were downregulated[10]. Furthermore, the genes encoding MYC and cyclin D1, which had previously been associated with cell transformation in pseudohypoxic renal cancer[34], were expressed at higher levels in *HIF2A*-mutants compared with pheochromocytomas and paragangliomas without these mutations[10]. Together, these initial findings suggest that mutations in *HIF2A* may confer a more aggressive phenotype to pheochromocytomas and paragangliomas, although these observations remain to be confirmed in larger cohorts.

Together, mutations of *VHL*, SDH and *HIF2A* account for most tumours with pseudohypoxic profiles. Nevertheless, a sizeable proportion of pseudohypoxic tumours do not carry mutations of these genes, and it is conceivable that other components of the hypoxic response might be aberrant in these cases, although novel mutant genes in these pathways have only been rarely identified[10,84–87] (TABLE 1).

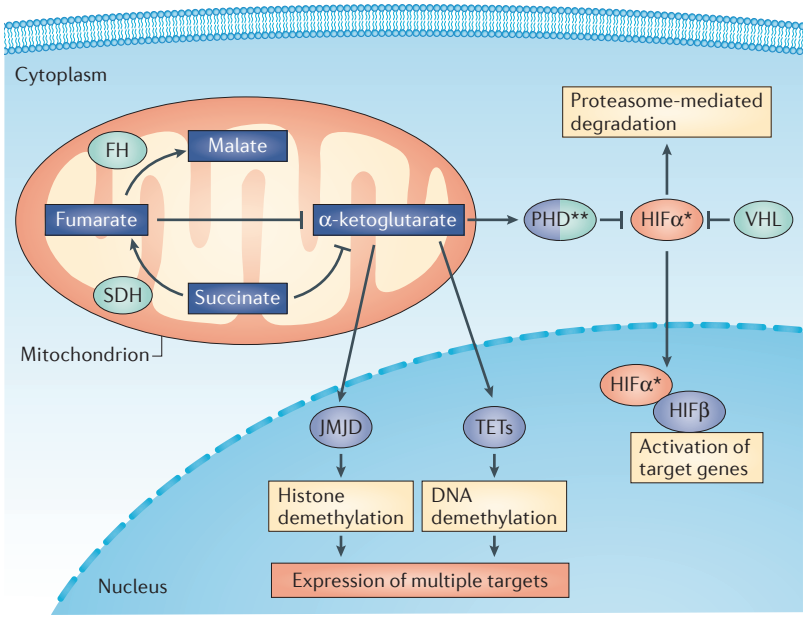

Figure 2 | **Cluster 1 mutations in paraganglial tumours.** Mutations in von Hippel–Lindau (*VHL*), succinate dehydrogenase (SDH) and hypoxia-inducible factor 2A (*HIF2A*) and at least in one case each in prolyl hydroxylase domain-containing protein 2 (*PHD2*) and in fumarate hydratase (*FH*) occur in pheochromocytomas and paragangliomas and can lead to increased activation of HIF. HIF1α or HIF2α subunits heterodimerize with HIF1β and transactivate multiple target genes involved in angiogenesis, metabolism and cell growth. *HIF2A* mutations lead to activated HIF-induced downstream transcription and resistance to degradation. *VHL* mutations impair proteasome-mediated HIF degradation. Mutations of SDH and *FH* result in the accumulation of their respective substrates, succinate and fumarate, which competitively inhibit α-ketoglutarate (αKG) for activation of various classes of αKG-dependent dioxygenases (shown in blue), including PHDs that promote HIF degradation, Jumonji (JMJ)-related histone demethylases (JMJDs), which demethylate histones; and the TET family of DNA hydroxylases (TETs), which demethylate DNA. Thus, inhibition of αKG-dependent dioxygenases collectively leads to HIF activation and global hypermethylation of target genes. HIF can also directly regulate the activity of some JMJ demethylases. Proteins with mutations that lead to loss of function are shown in green and proteins with activating mutations are shown in red. HIFα* represents either HIF1α or HIF2α, but only HIF2α is mutated in pheochromocytomas. PHD** represents PHD1, PHD2 and PHD3 (one paraganglioma has been reported with a PHD2 mutation) and is coloured in both green (loss-of-function mutation) and blue (αKG-target enzyme).

Cluster 2: the kinase signalling subgroup

The second transcription subgroup of pheochromocytomas and paragangliomas, known as cluster 2, which includes tumours with mutations in *RET*, *NF1*, *MAX* and *TMEM127* is discussed below (FIG. 3).

Box 1 | **HIF1α and HIF2α distinctions and metabolic effects**

Despite their high structural and functional homology, many features set these two isoforms apart. Hypoxia-inducible factor 1α (HIF1α) preferentially drives the expression of genes that are important for apoptotic and glycolytic pathways, whereas HIF2α activates genes that are involved in cell proliferation and angiogenesis[26,27]. Of particular relevance among these oncogenic targets are growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor-α (TGFα), which can promote cellular proliferation and the formation of new vessels. Another class of HIF targets that favours cellular adaptation to rapid proliferation is comprised of metabolic enzymes that coordinate energy synthesis[146]. In particular, enzymes of the glycolytic pathway, including glucose uptake and transport enzymes (solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1; also known as GLUT1)), hexokinase and glycerophosphate kinase are induced and lead to increased production of ATP and lactate. Through other targets, such as pyruvate kinase 1 (PDK1), HIF inhibits mitochondrial oxidative phosphorylation[147,148]. Preferential glycolysis over mitochondrial respiration for ATP synthesis has long been known as the Warburg effect, and is considered a hallmark of cancers[146,149]. Upregulation of multiple glycolytic enzymes also results in the activation of additional routes, including the pentose phosphate pathway, which supports the synthesis of macromolecules (such as nucleotides, lipids and amino acids) that are required for the increased demands of a highly proliferative tumour environment[149].

RET overactivation. Over the years, the study of *RET* has provided, at the clinical translational level, a model for genetic-based disease management, and, at the molecular level, has contributed to uncovering the oncogenic effects of kinase receptor activation. Germline gain-of-function *RET* mutations predispose to MEN2, subtype A (MEN2A) and MEN2B, autosomal dominant inherited syndromes characterized by pheochromocytoma and medullary thyroid cancer[88]. In addition, somatic *RET* mutations are detected in approximately 5% of sporadic pheochromocytomas and paragangliomas[12]. *RET* encodes a transmembrane receptor tyrosine kinase that is necessary for neural crest development[89]. RET activation requires ligand-dependent dimerization and autophosphorylation, a process that involves the binding of RET to the ligand, glial cell line-derived neurotrophic factor, in complex with a glycosyl-phosphatidylinositol (GPI)-anchored co-receptor (GFRα)[90].

The discrete distribution of activating *RET* mutations in specific codons of the gene dictates both the molecular and clinical outcome. This striking genotype–phenotype relationship formed the basis for genetic-based treatment recommendations of mutation carriers and has been discussed extensively elsewhere[14,91–95]. MEN2A-type *RET* mutations occur in the extracellular domain of RET and cause ligand-independent homodimerization and aberrant activation of PI3K–AKT, RAS, p38 MAPK and JUN N-terminal kinase pathways, resulting in the stimulation of cell growth, differentiation and survival[96,97] (FIG. 3). Within the group of MEN2A-related mutations, the specific codon also influences the level of mutant RET expression at the cell surface, which contributes to signal variability[96]. MEN2B-causing mutations, by contrast, target only a few codons that affect the catalytic site of the kinase and that lead to the loss of substrate specificity[97]. A knock-in mouse model of the most prevalent MEN2B mutation faithfully reproduced the human disease with high penetrance of pheochromocytomas[98]. Notably, no developmental defect, which may have been predicted as a result of abnormal substrate specificity of mutant RET, was detected in these animals[98]. RET, similar to other kinase receptors, is internalized and processed through the endosome, and this cycle influences the duration and specificity of its downstream signalling[99,100]. Although differential endosomal processing of mutant RET can occur in inactivating *RET* variants causative of the congenital colon disorder Hirschsprung's disease, aberrant endosomal processing has not been detected in activating pheochromocytoma- and paraganglioma-related *RET* mutations[101].

RET-activating mutations have spurred on the use of the kinase inhibitor vandetanib for the thyroid component of the disease[95]. However, pheochromocytomas are rarely metastatic in MEN2 syndromes[102] and their response to this inhibitor has not been tested.

Germline and somatic NF1 mutations. Germline mutations in *NF1* cause neurofibromatosis type 1, a frequently tumour-prone disorder (TABLE 1), in which as many as 5% of patients can develop pheochromocytomas or paragangliomas[103,104]. *NF1* encodes neurofibromin, a GTPase-activating protein (GAP) that functions as a tumour suppressor by promoting the conversion of the active, GTP-bound RAS GTPase to the inactive, guanosine diphosphatase (GDP)-bound RAS form, thereby terminating RAS signalling[105] (FIG. 3). RAS is a major oncogene in human malignancies (BOX 2) and the effects of inactivated NF1 generally reflect constitutively active RAS[106–108]. *Nf1*-null mice develop pheochromocytomas with high penetrance, and these tumours overexpress many genes responsible for the early development of the central and peripheral nervous systems, including RET[109]. Similar transcription patterns are seen in human pheochromocytomas with germline *NF1* mutations[11,12]. mTOR is a crucial downstream signal of both RAS and RET pathways, and is aberrantly activated in *NF1*-deficient malignant peripheral nerve sheath tumours[110], pheochromocytomas and paragangliomas[111].

Somatic *NF1* mutations were recently reported in 20–25% of sporadic pheochromocytomas, the most prevalent genetic lesion to have been found in pheochromocytomas and paragangliomas so far[7,9]. Similar to the germline mutations, these somatic variants were predominantly truncating and were often accompanied by the loss of the wild-type allele in the tumour[7]. In a rare violation of the 'mutual exclusivity rule' of susceptibility mutations in pheochromocytomas and paragangliomas, in a few cases a somatic *NF1* mutation was found in tumours carrying a somatic *RET* or a somatic *VHL* mutation[7]. These cases may either represent independent subclonal populations within the respective tumours or they may indicate that intra-cluster redundancy (*NF1* and *RET*) or inter-cluster cooperation (*VHL* and *NF1*) might confer a selective advantage to the target cell. These hypotheses can be tested in future studies by addressing downstream effects of multiple mutations, when compared with single mutations, of classic susceptibility pathways. Similarly, it

Familial erythrocytosis type 4
A specific congenital form of polycythemia caused by mutations in the *HIF2A* gene.

remains unclear what precise proportion of the 'sporadic' tumours carrying somatic *NF1* mutations do not have a susceptibility variant in another of the predisposing genes. These questions should be resolved by large-scale sequencing analyses.

MAX inactivation. Germline *MAX* mutations were discovered in patients with familial pheochromocytoma who lacked a mutation in other susceptibility genes[112]. More recently, analysis of an extensive patient cohort also revealed somatic *MAX* mutations in sporadic tumours[8].

MAX is a low abundance basic helix–loop–helix (bHLH) leucine zipper domain-containing protein that is predominantly found in complex with the MYC transcription factor, a common oncogene in many human cancers[113]. MYC–MAX heterodimers bind to E-box sequences in the promoters of more than 1,000 target genes that encode proteins with a wide range of cellular functions, including metabolism, growth and angiogenesis[113] (FIG. 3). MAX can also form complexes with other related bHLH leucine zipper transcription factors of the MXD1, MNT and MGA families, which oppose MYC-mediated activation by competing for the same E-box sequences and repressing the transcription of target genes that can ultimately lead to the inhibition of cell growth and/or the promotion of terminal differentiation[114]. A balance between MAX complexes with MYC and MAX complexes with MYC repressors dictates the output of transcription of E box-containing genes as a result of either activation or repression[113].

Despite the presumed activating role of the MAX–MYC interaction, the predominantly truncating nature of the mutations detected in patients with loss of the wild-type allele in the respective tumours challenged this paradigm by suggesting that MAX function is lost in pheochromocytomas[112]. Most of the mutations targeted highly conserved amino acids within the bHLH leucine zipper domain, which is responsible for protein–protein interactions and DNA binding, suggesting that they affect binding to MYC and to other MAX dimerization partners[8]. Although the mechanism through which *MAX* mutations cause pheochromocytomas and paragangliomas remains to be precisely defined it has been proposed that these tumours have increased transcription of MYC target genes[112]. In the rat pheochromocytoma cell line PC12, a common model for studies of neural differentiation, MAX is partially deleted and its reintroduction results in cell growth arrest, supporting a role for MAX in repressing MYC oncogenic effects in paraganglial cells[114,115]. An initial suggestion of increased malignancy of MAX-mutant pheochromocytomas and paragangliomas[112] could not be confirmed in a larger study[8], so it is unclear whether MAX mutation equates to the negative prognostic effect of increased MYC dosage that is well-documented in neuroblastomas, which are paediatric tumours of sympathetic neural crest origin[116].

An interplay between MYC-mediated oncogenesis and protein translation activation by mTOR-mediated phosphorylation of 4EBP1 was recently uncovered in lymphomas[117]. A similar link has not yet been examined in pheochromocytomas and paragangliomas. However, if MAX-mutant pheochromocytomas and paragangliomas are proven to be MYC-driven, the potential association between MYC and mTOR might explain why MAX-mutant tumours belong to cluster 2, along with *RET* and *NF1* mutants, which are known to activate mTOR signalling. Furthermore, if, like lymphomas, MAX-mutant tumours become addicted to mTOR-dependent phosphorylation of 4EBP1, this can offer an opportunity for pharmacological intervention.

Because MAX interacts with other proteins, including the MYC antagonists MXD1, MXI1 and MGA, it remains to be defined how MAX mutation might affect those complexes or influence the transcription output of E-box-containing genes. E-box elements are present in the promoters of multiple genes, including other pheochromocytoma susceptibility genes[118]. Determining whether MAX regulates the transcription of these genes may provide another level of modulation of their function that might affect tumour development.

TMEM127 mutations. Truncating or missense germline *TMEM127* mutations were identified in patients with pheochromocytoma in a pattern consistent with a classic tumour suppressor gene[111]. TMEM127 is a transmembrane protein of unknown function with three membrane-spanning domains. Since the original report, more than 30 mutations have been identified in *TMEM127*, 60% of which result in a truncated product or which predominantly target one of the transmembrane regions of the protein[119–123]. Although all variants were detected in germline DNA, less than 20% of patients carrying a *TMEM127* mutation report a family history of pheochromocytomas, suggesting low penetrance of the mutant alleles[124]. Germline *TMEM127* mutations were also detected in rare cases of renal cell carcinoma[125]. TMEM127 has broad tissue expression and the recombinant protein localizes to the plasma membrane and multiple components

Box 2 | **Other pheochromocytoma and paraganglioma genes**

Germline mutations in *KIF1B*, which functions in a JUN- and prolyl hydroxylase domain-containing protein 3 (PHD3)-dependent apoptosis pathway that occurs physiologically in sympathetic lineage precursor cells during development, were found in rare cases of pheochromocytomas[150,151]. This pathway, which is hypoxia-inducible factor (HIF)-independent, is disrupted by von Hippel–Lindau (VHL) type 2C mutants, and also by mutations in *SDHD* (succinate dehydrogenase complex, subunit D), neurofibromin 1 (*NF1*) and *RET* and has been proposed to account for the high heritability of pheochromocytomas and paragangliomas[134].

Somatic mutations of the *HRAS* gene, one of the most frequently disrupted genes in human cancers, were recently detected in four sporadic pheochromocytomas or paragangliomas by exome sequencing[135]. The mutations targeted two of the most commonly affected HRAS codons in other cancers and are predicted to activate signalling downstream of the RAS–MAPK pathway. These findings have yet to be replicated in other series, but a similar *HRAS* mutation in one pheochromocytoma is listed in the Catalogue of somatic mutations in cancer (COSMIC) database of human cancer mutations[152,153]. Recently, a germline mutation in the *BAP1* (BRCA1-associated protein 1) gene, which has been implicated in the development of both renal cell carcinomas and uveal melanomas, was detected in an individual with paraganglioma from a family with multiple cancers[154]. Although it remains unclear whether the tumour was part of the clinical spectrum of the syndrome in this family, loss of heterozygosity of the wild-type *BAP1* allele was found in the paraganglioma, in support of the inactivation of this gene. A direct link between pheochromocytomas and paragangliomas and BAP1 will require further investigation.

Box 3 | **Inheritance mode in pheochromocytomas and paragangliomas**

All inherited syndromes and familial forms of pheochromocytomas and paragangliomas with a detectable mutation are transmitted through an autosomal dominant trait. In three of these syndromes — familial paraganglioma syndrome type 1 (PGL1), which is caused by mutations in *SDHD* (succinate dehydrogenase complex, subunit D); PGL2, which is due to *SDHAF2* (succinate dehydrogenase complex assembly factor 2) mutations; and familial cases related to *MAX* mutations — there is a parent-of-origin preference in which the disease only manifests when the mutation is inherited from the paternal allele. The mechanism underlying the paternal transmission is not entirely clear and cannot be fully explained by classic imprinting[155]. Two alternative models have been proposed to explain the preferential paternal transmission. The Hensen model suggests that in cases in which *SDHD*, and possibly *SDHAF2* (located on the same chromosome) are mutated, the maternal allele is lost together with a putative, maternally expressed tumour suppressor gene, while the paternal allele is consistently retained[155]. The rare instances of maternal transmission of the disease would require an additional recombination event between the *SDHD* gene and the putative associated tumour suppressor for the disease to be manifest (equivalent to a third-hit model, as opposed to the two-hit phenomenon of classic tumour suppressor genes). A second model suggests that the disease transmission is the result of quantitative imprinting; that is, differential dosage of maternal and paternal alleles on a tissue-specific basis[156]. The paternal transmission of *MAX*-related tumours is not yet confirmed[112]. Regardless of the mechanism, the parent-of-origin effect leads to occasional generation skipping of the disease, which may contribute to the low rate of positive family history reported in these syndromes.

The co-association of hypoxia-inducible factor 2A (*HIF2A*) mutations with multiple paragangliomas and/or pheochromocytomas, polycythemia and somatostatinomas is highly suggestive of mosaicism[19,20,159]. Mosaicism occurs when a mutation takes place at the post-zygotic stage and the tissues affected can be variable. When germinal cells are targeted, the mutation can be transmitted to the offspring, similar to a classic germline mutation, and thus can affect patient counselling[157]. Mosaicism occurs in various disorders, including neurofibromatosis type 1 (REFS 157,158). Other modes of transmission may occur in pheochromocytoma and paraganglioma cases at high risk of inheritability without a detectable mutation, although such alternative forms have not yet been clearly documented.

of the endosome machinery, including early, late and recycling endosome, Golgi complex and lysosome[111]. This distribution is sensitive to variations in pH and nutrients[111]. The mutant *TMEM127* transcript is markedly downregulated in tumour samples and, when expressed ectopically, truncated mutants are either undetectable or detected at very low levels. Most mutants become diffusely distributed in the cytoplasm, suggesting that localization of TMEM127 to endomembrane pools is important for its tumour suppressor function[124]. However, the subcellular distribution of some mutants has not yet been mapped. Given the increasingly recognized contribution of endosomal regulation to cancer[126], this information could determine, for example, whether distinct mutations differentially target the various steps of the endocytic process.

TMEM127-mutant pheochromocytomas have a transcription profile that resembles that of tumours with mutations in *RET* and *NF1* genes, reflecting overlapping signals[111]. This association led to the detection of increased mTOR target phosphorylation both in human *TMEM127*-mutant pheochromocytomas and in cell lines depleted of TMEM127. Similar findings in cells from a recently developed mouse model of *Tmem127* inactivation further support a link between TMEM127 and mTOR activation *in vivo*. In this model, loss of TMEM127 was shown to disrupt the early-to-late endosomal transition and to enhance lysosomal biogenesis. These changes affect mTOR distribution explaining, at least in part, the effect of TMEM127 on mTOR signalling, by mechanisms that remain to be determined[125].

Interestingly, HIF activation can also promote cellular proliferation by at least two mechanisms that involve endocytosis. In *VHL*-null renal cancer cells, increased HIF activation can disrupt endosomal trafficking by suppression of an early endosomal component protein, rabaptin 5, and can result in the accumulation and activation of growth factor receptors[127]. In this model, deficient assembly of the early endosome results in the deceleration of epidermal growth factor receptor (EGFR) traffic through the late endosome and lysosome, leading to the accumulation of undegraded EGFR. The second mechanism involves HIF-mediated transcription of *CAV1*, the gene encoding caveolin, a component of caveolae membranes involved in receptor-independent endocytosis. Increased expression of caveolin favours the accumulation of EGFR in caveolae and engagement of the receptor through dimerization in a ligand-independent manner[128]. In both instances, HIF-dependent endosomal disruption enhances growth factor availability and augments downstream signalling[127,128]. Thus, precisely defining how TMEM127 functions within the endosome and how mutations can affect this balance could offer insights into the effect endosomal trafficking disruption might have on the development of pheochromocytomas.

Cluster 1 and cluster 2 overlap. Although cluster 1-related genes are discussed separately from cluster 2-type mutations above, several of the pathways activated in these two major groups are not unlinked. mTOR can activate HIF[129], and MYC cooperates with HIF2α in oncogenesis[130]. Activation of both mTOR[131] and MYC[37] can increase glycolysis through the transcription of glycolytic enzymes, which is also a fingerprint of HIF activation[132]. Interestingly, although pheochromocytomas and paragangliomas carrying *MAX* mutations align with cluster 2 and do not have a preponderant pseudohypoxic profile, they share some characteristics with HIF-activated tumours, including predominant noradrenaline secretion and low expression of

phenylethanolamine *N*-methyltransferase (PNMT; which catalyses the conversion of noradrenaline to adrenaline)[8]. It is still unknown whether mutant MAX can affect HIF2α–MYC interplay, but the multiple levels of crosstalk between HIF and MYC suggest that intersections between these metabolically engaged signals may be reflected by the biology of the mutant pheochromocytomas and paragangliomas. The fact that many pheochromocytoma and paraganglioma susceptibility genes have an effect on metabolism is further underscored by the upregulation of fatty acids, pyruvate and RNA metabolism pathways determined by gene-set enrichment analysis of *TMEM127*-mutant pheochromocytomas[111]. These functions have been related to mTOR activation[131,133], and their investigation in pheochromocytoma and paraganglioma models might open new avenues for exploring the interaction between TMEM127 and cellular metabolism.

Remaining gaps

Despite the advances of the past decade, many questions remain unanswered in the field of pheochromocytomas and paragangliomas. Some of these gaps are briefly discussed below and it is expected that large-scale genomic and epigenomic efforts that are currently under way will shed some light onto these open areas.

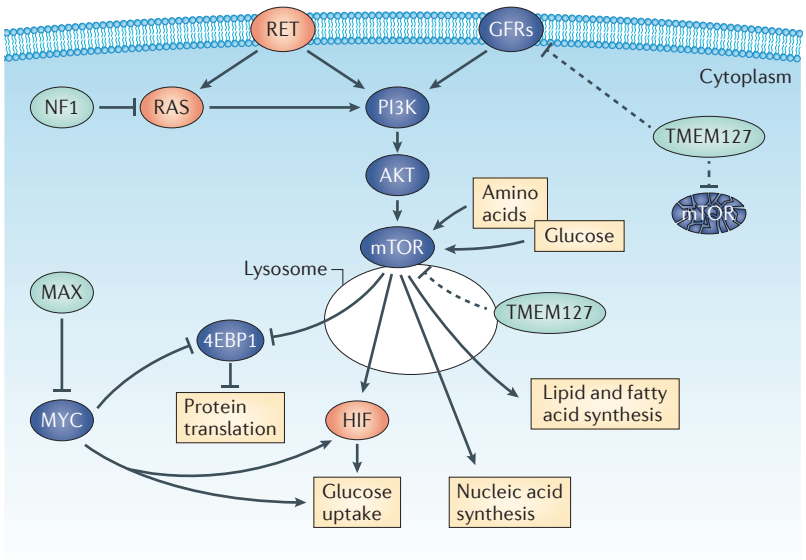

Figure 3 | **Pheochromocytoma and paraganglioma susceptibility genes in cluster 2.** RET, neurofibromin 1 (NF1), transmembrane protein 127 (TMEM127), MYC-associated factor X (MAX) and, more recently HRAS, when mutated, have been shown to lead to increased pro-growth signalling involving the receptor tyrosine kinase and downstream pathways, including mTOR and MYC. RET and other growth factor kinase receptor (GFRs), when activated, are internalized by endosomes (not shown) and initiate a cascade of events that lead to the activation of RAS and PI3K–AKT downstream signals including mTOR activation. mTOR is translocated from the cytoplasm to the lysosome to become activated. Active mTOR regulates cell growth through the synthesis of macromolecules, including protein (through inhibition of 4EBP1), nucleic acids, lipids and fatty acids, and increased glucose uptake (through hypoxia-inducible factor (HIF) activation). MYC binds to MAX to regulate transcription of multiple genes. In some cancers, MYC cooperates with mTOR to increase protein translation by inhibition of 4EBP1, and with HIF to increase glucose uptake and glycolysis. These pathways are held in check by various proteins: NF1, which inhibits RAS; MAX, which inhibits MYC; and TMEM127, which may inhibit mTOR (shown by dashed arrows to reflect the unclear mechanisms underlying this interaction). Multiple components of these pathways have been omitted for simplicity. Genes and proteins with activating mutations are shown in red and those with loss-of-function mutations are shown in green.

Other driver events. The actual mutation load of individual pheochromocytomas and paragangliomas is unknown. Until recently, the predominance of germline driver genetic events supported the concept that a defect during the development of precursor cells was a key driver of pheochromocytoma and paraganglioma tumorigenesis[134]. However, the recent identification of somatic mutations in these tumours[7,9,10,17,18,20,135], and co-occurrence of germline and somatic mutations[7] has brought to light alternative modes of tumour pathogenesis. The fact that overlapping areas of genomic loss or gain, which may contain mutations in oncogenes or tumour suppressor genes, are shared by pheochromocytomas and paragangliomas of distinct genetic backgrounds, and across transcriptional cluster boundaries[12,136] suggest that co-segregation of susceptibility mutations may confer a growth advantage to these tumours. Given that some tumours with an undefined molecular basis share the expression pattern of SDH-mutant pheochromocytomas and paragangliomas, it is possible that a combination of epigenetic and genetic disruptions may be implicated in the pathogenesis of additional tumours. Furthermore, recent evidence of frequent pathogenic mutations of promoter regions (telomerase gene) in melanomas[137] suggests that, to achieve a deeper understanding of pheochromocytomas and paragangliomas, sequencing efforts will have to extend beyond protein-encoding boundaries of the genome.

Malignancy markers. Malignancy in pheochromocytomas and paragangliomas can only be defined in advanced stages, and the inability to predict tumour behaviour does not allow for optimal therapeutic planning[4]. The clinical use of kinase inhibitors was initiated to capitalize on the aberrant angiogenesis and mTOR activation in primary samples or cells[42,138]. Results from smaller series have suggested some benefit with sunitinib[139], but not with everolimus[140], and will need to be reassessed in larger, prospective cohorts, especially in view of the fairly favourable short-term outcome of patients who are treatment naive[141].

Currently, mutations of *SDHB*, but not other subunits of SDH, are the strongest indicators of malignancy in pheochromocytomas and paragangliomas[142]. The mechanism behind this specificity is poorly understood, but a quantitative epigenetic switch, more pronounced in *SDHB* mutants than in other SDH-mutated tumours, was recently proposed to explain the increased malignancy risk conferred by *SDHB* mutations[60]. Activation of epithelial-to-mesenchymal transition has also been reported in these tumours[143]. However, *SDHB* mutations explain only a small proportion of malignant pheochromocytomas and paragangliomas, and other markers of malignancy or recurrence have not been identified. A possibility that has not yet been tested, but that can be addressed by genome-wide sequencing studies, is that certain clusters of mutations in different genes, rather than single driver mutations, might segregate with

biologically aggressive tumours. Whether epigenetic, genetic or a combination of these events account for the aggressive outcome of some pheochromocytomas and paragangliomas, and whether this knowledge can eventually be brought into diagnostic settings for the predictive management of patients, will certainly be the object of intense scrutiny in future research.

Mutational signatures. There has been renewed interest in defining specific mutational patterns that can reveal the effect of exogenous cancer-inducing agents[144]. Determining whether pheochromocytomas and paragangliomas carry unique signatures that might reveal specific vulnerabilities of sympathetic precursor cells could reveal aetiological agents in these tumours. Interestingly, it has long been noted that rats are uniquely prone to develop pheochromocytomas in response to a number of hormonal and other agents[145]. These observations suggest an influence of environmental cues on chromaffin cell growth.

Inherited tumours as relevant study models. Pheochromocytomas and paragangliomas have emerged from their position as 'niche' neoplasms to a more prominent status in oncology as a result of their power as 'engines' of tumour gene discovery, as a source of paradigm-shifting models in cancer biology and as pioneer examples of personalized medicine, in great part owing to their preeminent hereditary component. Moreover, mutations of pheochromocytoma and paraganglioma susceptibility genes in medullary thyroid carcinoma, renal cell carcinoma, GISTs and possibly pituitary adenomas, signal to a broader role of these genes in other cancers.

However, despite the relevance of inherited mutations in tumorigenesis, most mainstream genomic cancer studies have regarded germline mutations primarily as filters for the prioritization of somatic driver events, leaving the analysis of germline variants largely untapped. In addition to the rare, high-penetrance driver germline mutations discussed in this Review, the role of potentially more frequent, low-penetrance germline variants and how they may interact with somatic mutations to determine tumour phenotypes has not been explored and could yield much progress in our understanding of this and other neoplasms. In particular, germline and somatic interactions could have a major role in the extensive clinical variability observed among individuals carrying an identical germline mutation, including those within the same family. The time may be ripe for constitutive variants to enjoy the full attention of the cancer genomics community.

Final remarks

Pheochromocytomas and paragangliomas are the result of a combination of genetic lesions and epigenetic changes. Shared pathways are embedded into the main proliferative programme of each lesion, but the upstream driver mutation can determine the heterogeneity of the cellular and clinical outcome. A better understanding of this heterogeneity may enable the development and optimization of therapeutic strategies.

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Acknowledgements
The author is grateful to R. Aguiar for many suggestions and continuing support, to R. Clark for his critical reading of the manuscript, to members of the Dahia laboratory for their assistance and to the collaborators of the FP consortium for their contributions throughout the years. P.L.M.D. is supported by funds from the Cancer Prevention and Research Institute of Texas (CPRIT, RP110202), USA Department of Defense CDMRP (W81XWH-12-1-0508), Voelcker Fund and Greehey Children Cancer Research Institute (GCCRI).

Competing interests statement
The author declares no competing interests.

FURTHER INFORMATION
Catalogue of somatic mutations in cancer (COSMIC): http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
Multiple Endocrine Neoplasia type 2 (MEN2) and RET: http://www.arup.utah.edu/database/MEN2/MEN2_welcome.php
Pheochromocytoma and paraganglioma research support organization (PRESSOR): http://www.pressor.org/
TCA cycle gene mutation database (formerly SDH complex database): http://chromium.liacs.nl/lovd_sdh/home.php?action=switch_db
VHL tumor suppressor (mutations associated with Congenital Erythrocytosis) database: https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=VHL

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