Chapter 1

# Introduction

**The paraganglion system**

**Neoplasia of the paraganglion system**

**Genetics of paragangliomas**

**Molecular biology of paragangliomas**

**Historical notes**

**Outline of the thesis**

**References**

# 1. The paraganglion system

Paraganglia are small bodies of chromophil cell clusters associated with the ganglia of the autonomic nervous system. The paraganglion system consists of the adrenal medulla, the largest paraganglion in the human body, the sympathetic paraganglia, and the parasympathetic paraganglia[1,2]. The sympathetic paraganglia are associated with the ganglia of the paravertebral sympathetic trunk, the organ of Zuckerkandl, and the celiac, renal, suprarenal and hypogastric plexuses (figure 1)[2].

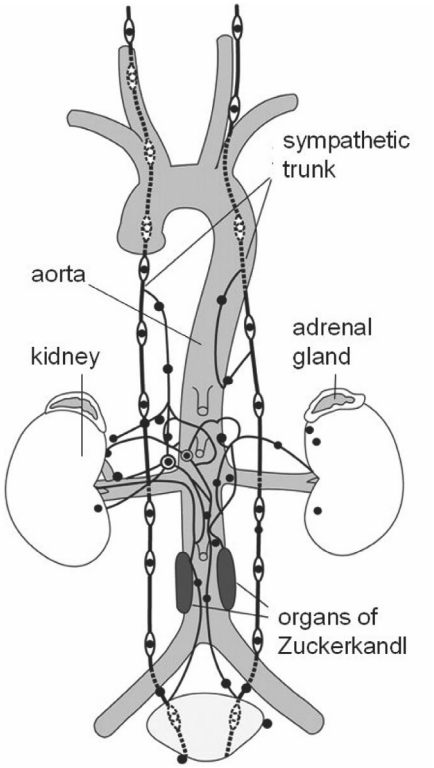



**Figure 1.** The adrenal medulla and extra-adrenal sympathetic paraganglia. Adapted from: Lee et al. Am. J. Roentgenol. 187 (2006) 492-504.

The parasympathetic paraganglia consist of the intravagal bodies and the branchiomeric paraganglia in the mediastinum and head and neck region, most notably located in the carotid bifurcation, the jugular foramen and on the promontory of the middle ear (figure 2)[2].

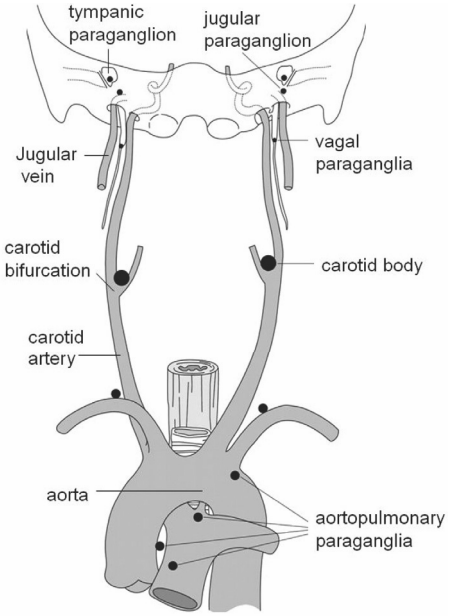



**Figure 2.** The parasympathetic branchiomeric paraganglia. Adapted from: Lee et al. Am. J. Roentgenol. 187 (2006) 492-504.

Paraganglia contain a parenchymal and a stromal component. The parenchymal component is of neuroectodermal origin. During embryogenesis, neuronal precursor cells migrate from the neural crest to locations along the cranial nerves, sympathetic trunk and greater blood vessels, where they develop into the paraganglionic type 1 or chief cells. The stromal component is of mesenchymal origin en contains the type 2 or sustentacular cells, as well as other stromal components such as blood vessels[3]. Type 1 and 2 cells form a specific configuration known as the “Zellballen”: small clusters of type 1 cells surrounded by type 2 cells and other stromal components (figure 3)[4].

The exact function of the paraganglion system is not fully known. The adrenal medulla, the inner part of the adrenal gland, produces the catecholamines adrenalin, noradrenalin and dopamine: hormones that regulate heart rate, blood pressure, metabolism, and cause vasoconstriction and bronchiole dilatation. The organs of Zuckerkandl are thought to be important regulators of the embryonic homeostasis and blood pressure through the production and release of catecholamines during early gestation, and they normally start to regress in the third trimester. The carotid and aortic bodies function as peripheral chemoreceptors sensitive to changes in arterial oxygen levels, and to a lesser degree also

to carbon dioxide levels and arterial pH. Arterial hypoxia, hypercapnia and acidosis cause excitation of the paraganglionic type 1 cells. This signal is relayed by the afferent fibers of the glossopharyngeal and vagal nerves to the central cardiorespiratory centers in the medulla oblongata, which regulate cardiac output and respiration (see paragraph 4.2.3: oxygen sensing at the carotid body, and paragraph 5.1: the discovery of the carotid body function)[5].

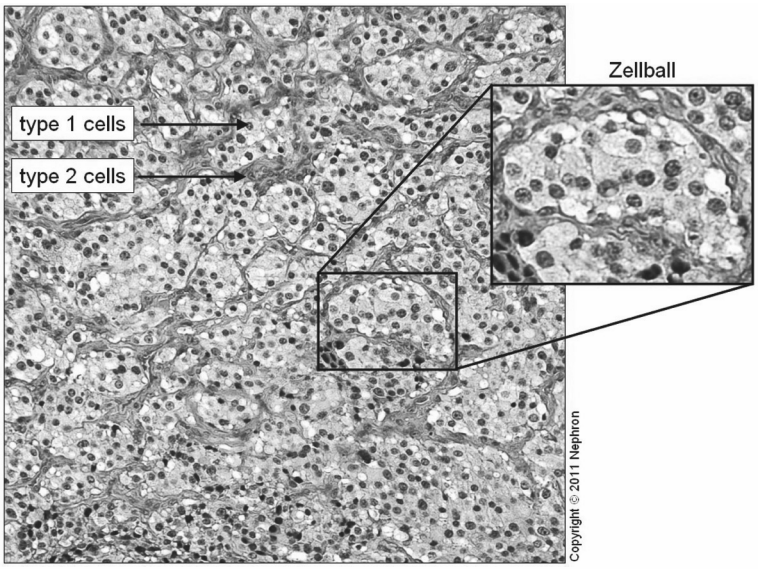



**Figure 3.** Microscopy of hematoxylin and eosin (H-E) stained carotid body paraganglioma tissue showing the type 1 and 2 cells in the classic Zellballen configuration. This characteristic architecture is usually preserved in the progression from normal paraganglion tissue to paraganglioma.

# 2. Neoplasia of the paraganglion system

The nomenclature of the neoplasia arising from the paraganglion system is equivocal and has changed over time. The terms 'chemodectomas', 'chromaffin tumors', 'glomus tumors', 'paragangliomas' and 'pheochromocytomas' have all been used interchangeably. The current classification according to the World Health Organization (WHO) designates tumors originating from the paraganglia in the head and neck region as 'paragangliomas', accompanied by the site of origin, i.e. 'carotid body paraganglioma'. The term 'pheochromocytoma' is reserved for tumors arising in the adrenal medulla, and 'extra-adrenal paraganglioma' for tumors developing in sympathetic paraganglia elsewhere in the retroperitoneal space, the abdomen, or the thorax[6].

However, some authors argue that the WHO classification is too refined, and as the distinctions between the subgroups are largely based on arbitrary conventions, consensus on the terminology of paraganglion tumors has thus far remained elusive. As a result, the term 'pheochromocytoma' is also used to describe all paraganglion tumors located outside of the head and neck region, or all paraganglion tumors located within the abdomen, or it is reserved for paraganglion tumors that secrete catecholamines and cause associated symptoms (see paragraph 2.3: 'functional paragangliomas')[6-8]. The use of the term 'glomus tumor' when referring to a head and neck paraganglioma, a remnant of the 19th century terminology for head and neck paraganglia, is quite persistent among physicians, but is better avoided because this term describes a completely different histological entity (namely a painful cutaneous tumor arising from neuromyoarterial glomus cells, characteristically located under the finger nails)[6,9,10].

Paragangliomas are usually slow growing and highly vascular tumors. The typical architecture of normal paraganglion tissue, the 'Zellballen' configuration consisting of type 1 and type 2 cells (figure 3), is usually maintained in the tumor, although in pheochromocytomas it may be less prominent[4,6,11]. It has been demonstrated that the tumorigenic component is formed by the type 1 or chief cells, and that the type 2 stromal cells show expansion under the influence of the type 1 cells[12].

### 2.1 Paragangliomas of the head and neck

Paragangliomas of the head and neck are rare tumors, representing approximately 0.6% of all head and neck neoplasms[13]. The incidence is estimated to be between 1:1.000.000 and 1:100.000, based on pooled data from Dutch pathology laboratories and surgical patients[4,14,15]. Due to the benign natural course of the disease, paragangliomas will not be surgically removed in a substantial proportion of the patients and it is therefore

likely that these figures represent an underestimation of the actual incidence[15,16]. Necroscopy rates for carotid body paragangliomas of 1:13.4000 to 1:3.860 also point towards a higher incidence[15,16]. The incidence of paragangliomas seems to be influenced by environmental factors that facilitate paraganglioma formation, such as high altitude, and by genetic factors, such as the regional clustering of paraganglioma patients due to a common hereditary trait, as can be seen in the Netherlands (see: 'genetics of paraganglioma', 'tumor biology of paragangliomas', and chapters 2, 3 and 4).

The majority of head and neck paragangliomas comprises of carotid body tumors, arising in the carotid bifurcation (approximately 61%). Approximately 19% is located along the vagal nerve, 12% are found in close relation to the jugular bulb or tympanic nerve, and 8% is located elsewhere in the head and neck region, most frequently along the larynx, the trachea or the aortic arch[4].

Symptoms of head and neck paragangliomas vary with the tumor localization. Most tumors are characterized by slow and expansive growth, but approximately 10-15% of the head and neck paragangliomas show a more aggressive, rapidly progressive behavior[17]. The most common symptom is a non-painful palpable neck mass or pharyngeal bulging. In addition, cranial nerve invasion or compression and subsequent dysfunction may occur, especially of the facial, glossopharyngeal, vagal, spinal accessory and hypoglossal nerves, because of their close anatomical relations with the jugulotympanic, vagal and carotid paraganglia. In case of tympanic or jugulotympanic tumors there may be conductive hearing loss and tinnitus, which is pulsatile in typical cases. Patients with functional paragangliomas can present with symptoms and signs of catecholamine excess (see paragraph 2.3: 'functional paragangliomas')[18]. A number of paragangliomas do not produce any clinical symptoms, and 6-16% are found as incidentalomas on imaging studies or through screening of paraganglioma families[18,19].

*Diagnosis*

The diagnosis of head and neck paragangliomas is based on the patient and family history, clinical investigation of the ears, the pharynx and the neck, biochemical screening for catecholamine excess (see paragraph 2.3: 'functional paragangliomas'), and radiology.

Detailed radiological examinations are essential for the diagnosis. The classic way of visualizing head and neck paragangliomas is digital subtraction angiography (DSA), which shows paragangliomas as highly vascular lesions. It is considered the gold standard in the diagnosis of small head and neck paragangliomas and for the identification of the vascular anatomy and main contributing blood vessels[20,21]. It is especially useful when surgery

is considered, because in addition to its role in the evaluation of the vascular structures, the angiography procedure may also be used to eliminate the main blood supply to the tumor or to perform a preoperative balloon occlusion test of the internal carotid artery (see paragraph 2.1: 'therapy')[20-22]. The disadvantages of the DSA technique are the need for catheterization (usually through the femoral artery) and the lack of visualization of the exact extension of the tumor and its relations to surrounding structures.

Nowadays DSA has largely been replaced by high resolution computed tomography (HRCT or CT) and magnetic resonance imaging (MRI). While both techniques are useful in assessing tumor extension, evaluating its anatomical relations, and detecting multiple paragangliomas within the head and neck region if present, MRI is the preferred modality because of its better visualization of soft tissues[20]. In addition, the CT imaging exposes the patient to radiation (albeit in a very low dose) and the contrast used might provoke catecholamine release in patients that are not pre-treated with alpha- or beta-blockers, although this complication was not found in recent studies[23-25]. The most accurate MRI technique in the detection of head and neck paragangliomas is a pre-and post-contrast enhanced 3D Time of Flight (TOF) MR angiography[20,26]. In both CT and MR imaging, it is essential to assess tumor extension in the axial as well as in coronal planes.

Functional imaging techniques like $^{131}$I-metaiodobenzylguanidine (MIBG) scintigraphy, $^{18}$F-fluorodopamine or $^{18}$F-fluorodihydroxyphenylalanine positron emission tomography (FDA-PET and FDOPA-PET, respectively) have a high specificity for paragangliomas because they detect abnormal isotope uptake by noradrenalin transporters in paraganglioma tissue[27,28]. They are useful when in doubt of the diagnosis and in whole-body screening for functional paragangliomas and pheochromocytomas, but a reduced sensitivity of MIBG and FDA-PET has been described in extra-adrenal and malignant paragangliomas[27-30]. $^{18}$F-fluorodeoxyglucose (FDG)-PET is efficient in whole-body screening for metabolically active tissue. As such, it is not very specific for paragangliomas or pheochromocytomas, but it is useful in screening for multiple tumors and has been shown to be a superior tool in the detection of paraganglioma metastases[27,28,30]. The lack of anatomical detail in the images, a disadvantage of PET imaging, can nowadays be overcome by combining PET and CT techniques, creating a single superposed image[20].

Definitive confirmation of the diagnosis is obtained by histopathology and the identification of the pathognomonic 'Zellballen' configuration within the tumor tissue. However, because of the high vascularity of these tumors and the risk of profuse bleeding upon biopsy, tissue samples for histopathology are rarely available prior to the surgical resection of the tumor. Nevertheless, if the origin of the lesion is uncertain and the diagnosis can not

be reliably made upon physical examination and imaging alone, one may consider fine needle aspiration biopsy (FNAB). Although the cytologic features of a paraganglioma are not very specific and cytology alone is therefore not sufficient for a reliable diagnosis of paraganglioma, the FNAB technique has been found to be safe and is sometimes required in order to rule out other types of malignancy[31,32].

*Classification*

Different classification systems exist for different primary paraganglioma sites in the head and neck region. For carotid body tumors, the classification according to Shamblin et al. is widely used[33]. Shamblin type I tumors are localized within the carotid bifurcation but do not involve the internal or external carotid artery; Shamblin type II tumors are adherent or partially surround one or both of these vessels; Shamblin type III tumors encase the internal and external carotid arteries, and extend to the hypoglossal nerve (figure 4). The Shamblin type can be evaluated preoperatively using CT or MRI imaging. The Shamblin type is positively correlated with the size of the tumor as carotid body tumors become more adherent to carotid vessels as they increase in diameter, and there is a correlation between the Shamblin classification and outcome after surgery, as cranial nerve injury (particularly to the vagal, the superior laryngeal, hypoglossal or facial nerve) is more likely to occur in larger tumors[33-35].

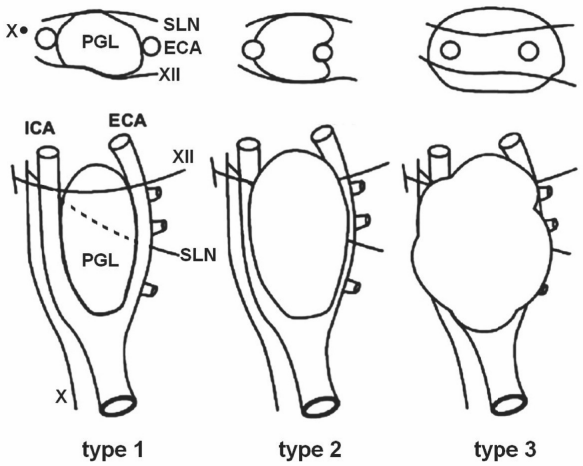



**Figure 4.** The classification of carotid body tumors according to Shamblin. The top row shows the axial views; the bottom row shows the sagittal views of Shamblin type 1, 2 and 3 paragangliomas (PGL). The classification is based on the relations of the tumor with the internal carotid artery (ICA), the external carotid artery (ECA), the vagal nerve (X), the hypoglossal nerve (XII) and the superior laryngeal nerve (SLN). Adapted from Arya et al. Am J Neuroradiol 29 (2008) 1349-1354.

Paragangliomas involving the temporal bone (tympanic and jugulotympanic tumors) are generally classified according to Fisch (table 1 and figure 5)[36,37]. This classification is primarily based on the extension of the tumor in the temporal bone and the involvement of the internal carotid artery, the jugular bulb, and the intracranial space. Jugulotympanic paragangliomas can be classified preoperatively using CT for Fisch type A and B paragangliomas, and a combination of CT and MRI for type C and D tumors. The Fisch type dictates the surgical approach necessary for tumor removal[37,38].

As of yet, no universally accepted system exists for the classification of vagal body tumors. The key features in the tumor description of vagal body tumors include the tumor size and its relations to the skull base and the internal and external carotid arteries[39].

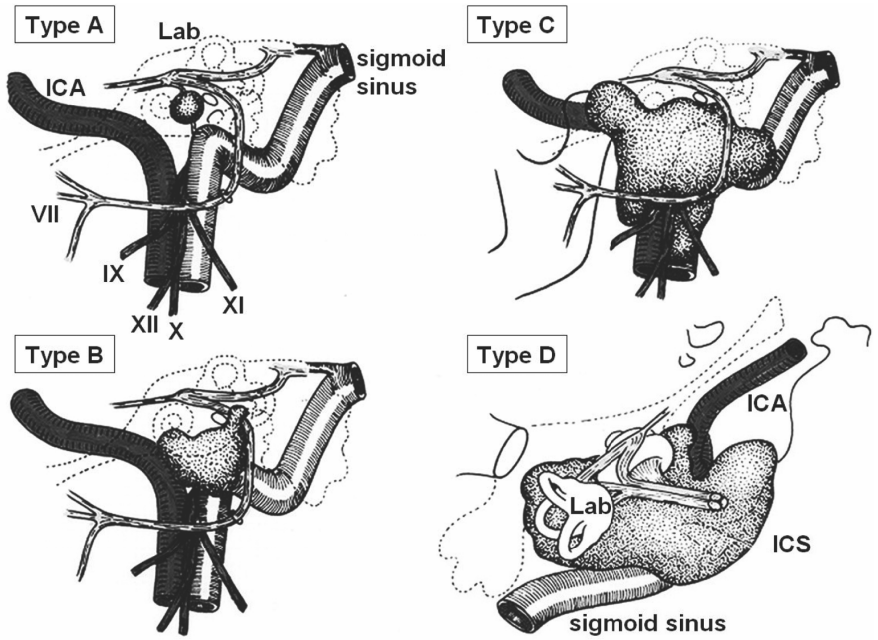



**Figure 5.** Schematic representation of the classification of temporal bone paragangliomas according to Fisch[36]. Type A, B, and C paragangliomas and their relations to the sigmoid sinus, the facial (VII), glossopharyngeal (XI), hypoglossal (XII), vagal (X) and spinal assessory (XI) nerves, the internal carotid artery (ICA) and the labyrinth (Lab) are shown in a sagittal view, type D is depicted in an axial view. Type A tympanic paragangliomas originate on the cochlear promontory and are limited to the mesotympanic space in the middle ear. Type B tympanic paragangliomas are limited to the middle ear and mastoid. Type C jugulotympanic paragangliomas show erosion of the bone covering the jugular bulb and extend along the ICA. Type D jugulotympanic paragangliomas extend into the intracranial space (ICS).

**Table 1.** The classification of temporal bone paragangliomas according to Fisch[36]. Combinations of C and D types are used to classify extended jugulotympanic paragangliomas.

| Type | Name | Extension |
|---|---|---|
| A | Tympanic paraganglioma | Limited to mesotympanum. No bone erosion. |
| B | | Limited to hypotympanum, mesotympanum and mastoid. No erosion of jugular bulb. |
| C | Jugulotympanic paraganglioma | Erosion of jugular bulb. Subclassification by degree of carotid canal erosion: |
| | C1 | no invasion of carotid canal |
| | C2 | invasion of vertical trajectory of carotid canal |
| | C3 | invasion of horizontal trajectory of carotid canal |
| | C4 | invasion of formane lacerum and cavernous sinus |
| D | | Intracranial extension, either extradural (De) or intradural (Di). |
| | De1 | intracranial extradural extension < 2cm |
| | De2 | intracranial extradural extension > 2cm |
| | Di1 | intracranial intradural extension < 2cm |
| | Di2 | intracranial intradural extension > 2cm |

*Therapy*

Today, there are 4 main strategies in the management of head and neck paragangliomas: surgical excision, embolization, radiotherapy, and watchful waiting.

The obvious benefit of surgical resection of paragangliomas is the removal of the tumor mass and the possibility of histological evaluation of the resection specimen, allowing for definitive confirmation of the diagnosis. Furthermore, future morbidity or progression to malignancy may be prevented. The surgical approach depends on the location of the paraganglioma within the head and neck region, the extension of the tumor, and its relations to adjacent structures. Surgery varies from relatively uncomplicated resections of Shamblin type 1 carotid body tumors and relatively straightforward middle ear and mastoid approaches in Fisch A and B type tympanic tumors, to extended head and neck surgery and infratemporal fossa approaches in Shamblin type 3 carotid body tumors and Fisch C and D type jugulotympanic tumors[34,35,37,38]. Due to the high vascularity of paragangliomas and their close anatomical relationships with the carotid artery, the jugular vein, multiple cranial nerves, and/or the skull base, there is a definite risk of surgical complications. Complete removal of the tumor is not always possible or may result in significant morbidity or even mortality, especially in larger tumors and tumors invading the skull base[15,21,37,40,41]. The cranial nerves that are most at risk when a surgical resection is performed are the glossopharyngeal, vagal, spinal assessory and

hypoglossal nerves in vagal and carotid body tumors; and the facial, vestibulocochlear, glossopharyngeal, vagal, spinal assessory and hypoglossal nerves in jugulotympanic tumors[35,37,39,41]. In vagal body tumors, the vagal nerve is almost always transected as vagal body tumors arise from its nodose ganglion[39,41]. The risk of peroperative cranial nerve injury is not always readily assessable preoperatively, as even in patients without preoperative cranial nerve deficit, infiltration or encasement of cranial nerves by paraganglioma tissue is present in 50% of the cases[42]. Cranial nerve deficit is especially incapacitating when cranial nerves are bilaterally affected, as this precludes the compensation of function from the contralateral side.

A possible additional complication of bilateral resections of carotid body paragangliomas specifically is the loss of the regulation of the hypoxic ventilatory response, resulting in immediate hypoventilation and respiratory acidosis (see paragraph 4.2.3: 'the hypoxia pathway')[43].

In larger vagal, carotid body and temporal bone paragangliomas, intraoperative control of the carotid arteries and jugular vein is compulsory, and grafting or sacrifice of these structures is sometimes necessary[41]. Whereas unilateral sacrifice of the jugular vein is generally well tolerated, bilateral resection or ligation may lead to elevated intracranial pressure and neurological sequelae[44,45]. If the need for ligation or partial resection of the internal carotid artery is anticipated, a preoperative intraluminal balloon occlusion test is recommended to evaluate the effects on the brain[20].

In order to minimize surgical difficulty and risk of uncontrollable bleeding, embolization of the main contributing blood vessels prior to the excision of the paraganglioma can be very helpful, especially in vagal and jugulotympanic tumors. In tympanic and carotid body tumors, the benefits of this procedure are not so clear[20,21,37,46-48]. Embolization is also performed as a primary palliative therapy for symptomatic or malignant paragangliomas as it may reduce tumor size, however the effects are almost always temporary, as alternative blood supply will develop and the tumor retains its potential to grow[49,50].

The third treatment option, radiotherapy, can be used as a primary treatment or as an adjuvant therapy after incomplete surgical resection of a paraganglioma[21,51]. As a single modality therapy, it has a much reduced risk of intraoperative bleeding and cranial nerve injury[21,52,53]. Because eradication of head and neck paragangliomas is not achieved by radiotherapy, the objective is local control of tumor growth, and one of the obvious drawbacks is therefore the persistence of the tumor mass[20]. Other disadvantages include the possible long-term effects of irradiation such as osteoradionecrosis of the skull base,

potential induction of malignancy, and an increased surgical difficulty and risk if resection proves to be necessary at a later stage[54,55]. Furthermore, there is evidence from histopathology that the tumor response to radiotherapy is unpredictable[56]. With the advent of stereotactic radiotherapy and 'gamma-knife' strategies, irradiation of adjacent normal tissue has been reduced while reported local tumor control rates (90-92%) are comparable to conventional radiotherapy[51,52,57]. However, as the natural course of most paragangliomas is characterized by no or very slow growth, it is difficult to ascertain whether a non-growing paraganglioma after radiotherapy is the result of tumor control by successful radiotherapy or due to the indolent natural course of the disease[20,39,58].

The fourth option in the management of head and neck paragangliomas consists of a policy of watchful waiting, also called 'wait and scan'. No intervention is performed, and tumor growth is monitored regularly with repeated MRI. Surgery or radiotherapy is undertaken only if there is evidence of tumor growth or impending complications. The disadvantage of this strategy is the persistence of the tumor and its potential to grow, however, most head and neck paragangliomas are characterized by slow growth, a substantial number of head and neck paragangliomas will not become symptomatic, and the effects of cranial nerve palsy are often better mediated if the paresis is slowly progressive due to tumor growth as opposed to sudden paralysis due to surgical injury[19,20,39,58].

Not surprisingly, the optimal treatment strategy for head and neck paragangliomas is subject of much debate in the literature[21,22,53,58,59]. The choice of treatment modality and timing require a multidisciplinary approach, and are tailored to the individual patient, depending on symptoms, tumor location, tumor stage, mutlifocality, catecholamine excess (see below), heredity and the causative gene mutation[15]. Surgical resection of paragangliomas is considered either when the tumor is small and total removal is not likely to cause significant cranial nerve injury and associated morbidity, or when cranial nerve deficit has already been caused by the tumor and complete resection is unlikely to cause additional problems. A rationale for surgical intervention is the anticipation of complications due to progression of tumor extension towards cranial nerves, the skull base, or the carotids[15,20,21]. Furthermore, surgical resection is the therapy of choice in functional paragangliomas (see below). Radiotherapy, stereotactic radiotherapy or 'gamma knife', although considered by some as the primary treatment of choice in all paragangliomas, are primarily used as a palliative treatment in malignant paraganglioma, or in progressive paragangliomas in which surgery is deemed to confer a high risk of significant morbidity[15,20,21,52]. A conservative treatment strategy consisting of closely monitoring the natural course of the disease without intervention (´wait and scan'), seems, at least initially, appropriate for many asymptomatic patients, elderly patients,

patients with multiple and bilateral paragangliomas, tumors with extensive temporal bone involvement and tumors caused by a gene mutation that is characterized by a mild disease phenotype[15,20,21,39].

### 2.2 Pheochromocytomas and extra-adrenal paragangliomas

Pheochromocytomas and extra-adrenal paragangliomas, together described as sympathetic paragangliomas, are tumors closely related to head and neck paragangliomas. Sympathetic paragangliomas are rare tumors, with an estimated incidence between 1:500.000 and 1:50.000[60,61]. About 80% of the sympathetic paragangliomas originate from the adrenal medulla and are called pheochromocytomas. The remaining 20%, called extra-adrenal paragangliomas, occur elsewhere in the sympathetic paraganglia, most frequently in the abdomen and pelvis, less frequently in the thorax[29]. Extra-adrenal paragangliomas more often progress to metastatic disease than either pheochromocytomas or head and neck paragangliomas [62].

The symptoms of pheochromocytomas are usually caused by the secretion of catecholamines or their metabolites by the tumor, and include hypertension (in about 60%) which may be fluctuating or sustained, paroxysmal palpitations, headache, agitation, excess sweating and pallor.

The diagnosis of pheochromocytomas and extra-adrenal paragangliomas is based on biochemical screening for catecholamine excess (see paragraph 2.3: 'functional paragangliomas') and radiology. Both abdominal CT and MRI are sensitive modalities for the detection of abdominal masses. As described above, some authors prefer MRI because no iodine containing contrast is needed to adequately visualize the tumor.

The mainstay of pheochromocytoma therapy is surgical resection. The preferred technique is a partial or cortical sparing adrenalectomy via a laparoscopic or retroperitoneoscopic approach, as this minimizes surgical risk and morbidity[63-66]. Bilateral endoscopic cortical sparing adrenalectomies should be considered in bilateral pheochromocytomas or in case of a genetic predisposition for developing bilateral disease, although there is debate as to whether partial adrenalectomy is associated with an increased long-term recurrence rate in hereditary cases[63,66-68]. Total adrenalectomy is indicated in malignant pheochromocytomas and sometimes unavoidable in benign pheochromocytomas, especially in large tumors or recurrent disease[66,68]. If performed bilaterally, total adrenalectomy carries the risk of potentially life-threatening post-operative Addisonian crises and necessitates lifelong corticoid supplementation therapy[66,69]. Adrenalectomy via an open laparotomy is nowadays rarely indicated, even in large tumors (i.e. tumors with a

diameter exceeding 6 cm)[70]. There is debate as to whether open procedures should be performed in malignant pheochromocytomas and extra-adrenal paragangliomas. Most authors agree that it is indicated in case of large malignant tumors, local invasion, or if resections of neighboring organs are required[66,71-73]. Because of the risk of catecholamine excess during, and catecholamine depletion after an adrenalectomy procedure, peri-operative treatment with α- and β- adrenoreceptor antagonists, calcium channel blockers and/or catecholamine synthesis-inhibitors is mandatory (see paragraph 2.3: 'functional paragangliomas').

**2.3 Functional paragangliomas**

A proportion of the neoplasia of the paraganglion system is 'functional', i.e. they secrete vasoactive catecholamines like dopamine, adrenalin and noradrenalin and/or their metabolites. Excess catecholamine secretion is a well-known feature of adrenal pheochromocytomas and extra-adrenal paragangliomas, but relatively rare in head and neck paragangliomas (1-5%)[18]. The majority of functional paragangliomas produce noradrenalin, a few secrete dopamine or adrenalin[74,75]. Catecholamine secreting tumors are best detected through the evaluation of the urine or plasma concentrations of metanephrine and normetanephrine, metabolites of catecholamines. Plasma free metanephrine measurements are the most accurate diagnostic tool, with a superior sensitivity (97-99%) and specificity (86-97%)[15,74,76,77]. The next best technique, 24-hours urinary metanephrine and normetanephrine measurements, has a comparable high sensitivity (96-97%) but lower specificity (45-82%)[15,74]. The latter is still widely used in the Netherlands due to better availability[15].

Biochemical screening should be performed if a patient's signs or symptoms indicate a functional paraganglioma, in case of a genetic risk for the development of paraganglioma-pheochromocytoma syndrome, and in all pheochromocytomas and extra-adrenal paragangliomas (see: 'genetics of paragangliomas')[15,27,29]. If catecholamine secretion is present in head and neck paragangliomas, it causes the same symptoms as it does in pheochromocytomas (hypertension, palpitations, headache, agitation, excess sweating and pallor). Prolonged exposure to high levels of catecholamines can result in hyperglycemia, electrolyte disturbances and cardiovascular complications such as cardiac hypertrophy, myocardial infarction or heart failure. Multiple organ failure, shock and sudden death by stroke or cardiac arrest due to catecholamine excess have been reported[29,78,79]. Because of these potentially life-threatening conditions, surgical excision is the treatment of choice in functional paragangliomas[15,27]. Peri-operative measures consisting of pre-operative volume expansion by intravenous saline, stringent intra-operative monitoring, and treatment with α- and β- ardrenoreceptor antagonists, calcium channel blockers and/

or catecholamine synthesis-inhibitors are compulsory to counter critical hypertensive crises and compensatory hypotensive episodes due to manipulation and removal of the tumor[29,65].

### 2.4 Malignancy

Most paragangliomas are benign tumors, i.e. they do not metastasize and are characterized by an expansive rather than an invasive growth pattern. However, some paragangliomas, especially those within the petrous bone, show erosion of the surrounding bone, some show microvascular invasion, and some do metastasize. As of yet, no definite histologic criteria for malignancy have been established in paragangliomas[6]. Even in malignant paragangliomas and their metastases, the well differentiated architecture of normal paraganglion tissue is usually maintained[4,11]. Factors such as a higher mitotic rate, tumor cell spindling, altered nuclear morphology, aberrant DNA-ploidy, necrosis, and capsular or microvascular invasion are reported to be more prevalent in malignant paragangliomas, but all are also found in benign paragangliomas[4,6,11]. Immunohistochemical markers such as Ki-67, Cyclin-D1, p53, p21, p27, BCL-2 and MDM-2 have been shown to be of little use in predicting malignant behavior in paragangliomas[6,11]. Malignancy in paragangliomas is therefore defined as the occurrence of metastatic paraganglioma cells in non-neuroendocrine tissue.

Paraganglioma metastases are most frequently confined to cervical lymph nodes (69%). Distant metastases are identified in 31% of malignant head and neck paragangliomas, and the distant predilection sites include bone, lung and liver[80].

Several studies have assessed clinical factors that may predict malignancy in paraganglioma patients. Features such as a young age at diagnosis, pain as an accompanying symptom, a rapidly enlarging tumor mass, a large tumor size, and a mediastinal or extra-adrenal abdominal tumor localization all seem to be associated with an increased risk of malignancy, but none of these features are proof of malignancy in themselves[11,81,82]. Tumors that secrete catecholamines may be malignant or benign in nature. There is some debate as to whether dopamine secretion is indicative of extra-adrenal tumor localization and malignancy, but recent studies show that dopamine secretion is not uncommon in benign head and neck paragangliomas (19-23%), and that it is not related to metastatic disease or outcome[75,83-85]. The risk of developing malignant paraganglioma or pheochromocytoma is however correlated with the causative gene defect (see: ‘genetics of paragangliomas’).

The management of malignant paragangliomas is challenging. More aggressive treatment strategies are aimed at eradication and/or control of tumor growth both at the primary and metastatic site. In case of head and neck paragangliomas with metastases limited to regional lymphe nodes, surgical resection of the primary tumor combined with a neck dissection is the treatment of choice if feasible. In this patient group, no clear beneficial effect of adjuvant radiotherapy has been found if resection margins are negative[80]. In case of incomplete resections, adjuvant therapy may consist of embolization, radiotherapy, systemic chemotherapy (with cyclophosphamide, vincristine and dacarbazine) or combinations thereof[80,86-88]. In case of incurable metastatic disease, palliative treatment strategies include surgical tumor debulking, embolization, pharmacological blocking of catecholamine secretion, palliative conventional radiotherapy, metabolic targeted radiotherapy with $^{131}$I-MIBG, and/or systemic chemotherapy[80,86-88]. A recent development is the advent of possible targeted molecular therapies. Currently, several are being investigated in patients with malignant paraganglioma and pheochromocytoma[71,72,89-93]. Promising results have been reported of temozolomide and thalidomide combination therapy, of sunitinib (a tyrosine kinase inhibitor), and of somatostatin analogues, but their effectiveness has not yet been validated by clinical trials[71;72;90-93]. As surgical resections confer a risk of surgical complications as mentioned above, and the non-surgical interventions can be complicated by bone marrow depression and a fatal sudden increase in catecholamine levels due to tumor necrosis, treatment of incurable metastatic disease should only be considered if the quality of life is threatened by symptoms caused by local tumor extension or catecholamine excess[86-88]. A policy of watchful waiting may be considered a viable option in patients with stable metastatic disease and mild symptoms[86-88].

Due to the rarity of malignant paragangliomas and the number of different strategies and regimens that have been applied over time, data on the outcome of these interventions are largely retrospective, not fully comparable, and often biased, and the lack of controlled prospective trials hampers the recommendation of specific therapies[80,86,87].

Without taking treatment strategies into account, patients with malignant head and neck paragangliomas have a reported overall five year survival rate of 55-60%[80,86,94]. Survival is greatly influenced by the site of the metastasis, as the five year survival rate of patients with metastatic disease limited to regional lymph nodes (77%) is significantly better than of those with distant metastasis (12%)[80]. Furthermore, survival seems to be influenced by the causative gene, as the five year survival rate after first metastasis is 37% in patients carrying a mutation in the *SDHB* gene, whereas it is 67% in the absence of *SDHB* mutations (see: 'genetics of paragangliomas')[94].

# 3. Genetics of paragangliomas

## 3.1 NF1, RET, and VHL

The knowledge of paraganglioma genetics has long been limited to mutations in genes causing neurofibromatosis type 1 (the *NF1* gene), multiple endocrine neoplasia (MEN) type 2a and 2b (the *RET* gene), and Von Hippel-Lindau syndrome (the *VHL* gene)[6]. Whereas pheochromocytomas are a well-known element of the tumor spectrum of these syndromes, head and neck paragangliomas caused by *NF1*, *RET* or *VHL* mutations are rare and almost never occur as the sole manifestation of the disease. *NF1*, *RET* or *VHL* mutations and their associated syndromes are discussed briefly with a focus on their relevance in head and neck paragangliomas and pheochromocytomas.

*NF1*

Neurofibromatosis type 1 (NF1) is caused by mutations in the *NF1* gene located on 17q11. It encodes the neurofibromin 1 protein, a negative regulator of the Ras intracellular signaling pathway which is involved in cell growth, differentiation and survival (table 2). NF1 is the most common tumor syndrome of the peripheral nervous system, with a prevalence of 1 in 3000[95]. The syndrome consists of cutaneous and peripheral nerve neurofibromas, and is also associated with gastrointestinal tumors, gliomas and myeloid leukemia. The estimated prevalence of pheochromocytomas in NF1 patients is 0.1- 6%, although necroscopy rates are higher (3-13%)[95]. As a rule, pheochromocytomas do not occur without other manifestations of neurofibromatosis, most often neurofibromas and café-au-lait spots on the skin[95;96]. The mean age at diagnosis of the first pheochromocytoma is approximately 41 years, bilateral adrenal involvement occurs in 27%, extra-adrenal localizations are infrequent (5%), and the malignancy rate is 6%[96]. Head and neck paragangliomas associated with NF1 have been reported but are extremely rare[97,98]. In a recent international study including 809 head and neck paraganglioma patients that were not linked to mutations in succinate dehydrogenase (see paragraph 3.2: 'the succinate dehydrogenase genes'), no NF1 mutations were identified[97,98].

*RET*

Mutations in the *RET* proto-oncogene (located on 10q11) cause MEN type 2 syndromes. *RET* encodes a receptor tyrosine kinase and functions as the receptor for extracellular signaling molecules of the glial cell line-derived neurotrophic factor (GDNF) family (table 2)[99]. MEN type 2 syndromes can be divided into MEN type 2a, characterized by the occurrence of medullary thyroid carcinoma, pheochromocytoma, and primary hyperparathyroidism, and MEN type 2b, characterized by medullary thyroid carcinoma, pheochromocytoma, mucosal neuroma and a marfanoid habitus[99]. In MEN type 2 syndromes, the risk of

developing a pheochromocytoma is high (50%), and if a pheochromocytoma is found, multifocal or bilateral tumors are common (50-80%), but extra-adrenal paragangliomas are rare (3%), as is pheochromocytoma malignancy (3%)[99,100]. *RET* mutation carriers seldom develop head and neck paragangliomas (in approximately 0.1%)[98].

*VHL*

Von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome caused by mutations in the *VHL* gene located on 3p25-26 (table 2). *VHL* encodes a subunit of the VHL ubiquitin ligase complex, a key component in the degradation of hypoxia inducible factor (HIF) 1α, 2α and 3α subunits (see paragraph 4.2.3: 'the hypoxia pathway', and figure 8)[101]. Specific homozygous recessive mutations in the *VHL* gene do not cause tumors, but a rare form of hereditary polycythemia called Chuvash syndrome[101]. Patients with the VHL tumor syndrome carry heterozygous mutations and the disease is inherited in an autosomal dominant way[101]. In these heterozygous *VHL* mutation carriers, *VHL* acts as a tumor suppressor gene, i.e. loss of the wild type allele is required for tumorigenesis. Mutations predispose to a variety of tumor types, including hemangioblastoma of the retina and central nervous system, clear cell renal carcinoma, neuroendocrine pancreatic tumors and endolymphatic sac tumors[95,101,102]. Pheochromocytomas are found in 20% of VHL patients, and the mean age at diagnosis of the pheochromocytoma is 28 years[95,102]. In the pediatric pheochromocytoma population, *VHL* mutations are the predominant cause of the disease (accounting for 40% of the cases)[95,102]. Most VHL-linked pheochromocytomas are benign and bilateral[95]. Head and neck paragangliomas are found in less than 1% of VHL cases, and *VHL* mutations account for approximately 2% of the head and neck paraganglioma population[98,103]. Almost all VHL-linked head and neck paraganglioma patients have additional manifestations and/or a positive family history of VHL disease[98].

## 3.2 The succinate dehydrogenase genes

In 2000, Baysal et al. in collaboration with the Paraganglioma research Group Leiden, discovered that mutations in succinate dehydrogenase subunit D (SDHD), a subunit of the mitochondrial succinate dehydrogenase complex (SDH), cause hereditary head and neck paraganglioma syndrome type 1 (PGL1)[104]. This breakthrough discovery initiated the identification of other SDH genes as the causes of the PGL2, PGL3 and PGL4 paraganglioma syndromes. Parts of this overview have been adapted from chapter 2, which reviews the current developments in paraganglioma genetics.

*SDHD*

The *SDHD* gene is located on the long arm of chromosome 11 (11q23). The mapping of its locus on 11q23 and its subsequent identification as the cause of PGL1 syndrome was greatly facilitated by the concentration of large PGL1 kindreds in the proximity of the city of Leiden, located in the western part of the Netherlands[104-107]. The discovery of *SDHD*, a nuclear gene encoding an anchoring subunit of SDH, was the first time that a mitochondrial protein was identified as a tumor suppressor (figure 7). It was furthermore the first protein with a role in the intermediary metabolism to be directly linked to tumorigenesis[104]. SDHD-linked paraganglioma syndrome is characterized by the formation of benign head and neck paragangliomas, and metastatic disease is rare (0-10%)[62,108-112]. SDHD-linked patients have a high risk of developing multiple paragangliomas (30-74%), and are also at risk of developing a concurrent pheochromocytoma (7-53%)[62,108-110,113]. The diagnosis is generally made in the third or fourth decade of life (mean age at diagnosis 25- 38 years)[62,108,109]. The penetrance of *SDHD* mutations is high upon paternal transmission (87-100%), although not all paraganglioma patients develop tumor-related symptoms[19,62,109,114-116]. Maternal transmission of disease is extremely rare (see paragraph 4.3: 'inheritance of head and neck paraganglioma syndromes')[117]. In the Netherlands, mutations in SDHD are the major cause of head and neck paragangliomas, probably due to the occurrence of multiple Dutch founder mutations (see also chapters 3 and 4)[118,119]. The incidence of SDHD mutations and the clinical characteristics of SDHD-linked paraganglioma syndrome in the Netherlands are further discussed in chapters 3, 4 and 5.

*SDHC*

In 2000, *SDHC*, located on chromosome 1 (1q23), encoding another SDH anchoring subunit, was found to be the causative tumor suppressor gene in paraganglioma syndrome PGL3 (figure 7)[120]. Mutations in *SDHC* are primarily associated with benign head and neck paragangliomas, although extra-adrenal paragangliomas, pheochromocytomas and malignancy have been reported in SDHC-linked cases[110,120-123]. The average age at diagnosis is 38-46 years[110,121]. Mutations in *SDHC* are a rare cause of paragangliomas, with only 19 index cases and 30 affected patients reported to date[124]. In the Netherlands, *SDHC* mutations represent less than 0.5% of the mutations found in SDH genes (chapter 3)[125]. The inheritance of SDHC-linked disease is autosomal dominant, and the penetrance of *SDHC* mutations is as yet unknown, but the very low incidence of SDHC-related paraganglioma suggests that it is incomplete.

*SDHB*

In 2001, *SDHB*, located on chromosome 1 (1p35-36.1), encoding the catalytic iron-sulfur SDH subunit, was linked to paraganglioma syndrome PGL4 (figure 7)[126]. It acts as a tumor suppressor gene and it has been shown to be the dominant cause of hereditary paraganglioma syndrome in many parts of the world[62,108]. SDHB-linked paraganglioma syndrome is characterized by a high rate of extra-adrenal paragangliomas (52-84%), while pheochromocytomas (18-28%) and head and neck paragangliomas (27-31%) are less frequently found[62,108,109]. The mean age at diagnosis is 30-37 years, and up to 38% of *SDHB* mutation carriers develop metastatic disease[62,103,108-110,121]. Most SDHB-linked tumors present with catecholamine excess; 10% of the tumors is biochemically silent or produces dopamine only[62,127]. The inheritance of SDHB-linked disease is autosomal dominant, and the penetrance of *SDHB* mutations is estimated to be 26-35%, much lower than that of *SDHD* mutations, which explains why *SDHB* mutations are more often found in isolated paraganglioma patients[128,129,129-131]. In the Netherlands, *SDHB* mutations seem to be remarkably uncommon, and account for only 3% of the head and neck paraganglioma patients and 6% of all SDH mutations (chapters 3 and 4)[111,125].

*SDHAF2*

The gene encoding succinate dehydrogenase assembly factor 2 (*SDHAF2*, formerly known as SDH5), is located on the long arm of chromosome 11 (11q13). In 2009, it was linked to head and neck paraganglioma syndrome PGL2 (table 2)[132]. *SDHAF2* acts as a tumor suppressor and does not encode a SDH subunit, but a co-factor related to the function of the SDHA flavoprotein subunit. The p.Gly78Arg missense mutation is the only pathogenic mutation in SDHAF2 known to date[133-135]. SDHAF2-linked paraganglioma syndrome is characterized by the formation of benign head and neck paragangliomas[133,135]. Most patients develop multiple head and neck tumors (70-91%), but no extra-adrenal paragangliomas, pheochromocytomas, or malignant paragangliomas have been reported in association with SDHAF2 mutations[111,133,135]. The mean age at diagnosis is 33-34 years[111,133]. SDHAF2-linked disease is characterized by the same parent-of-origin dependent inheritance pattern as SDHD-linked paraganglioma syndrome, i.e. no paragangliomas develop upon maternal transmission of the SDHAF2 mutation, whereas the risk of disease upon paternal transmission of the mutation is very high (88-100%) [132,133]. The SDHAF2 mutation is currently only found in a large Dutch paraganglioma kindred and an unrelated Spanish family[134]. In the Netherlands, it accounts for 7% of SDH mutation carriers, and approximately 4% of the head and neck paraganglioma patients (see chapters 3 and 4)[111,125].

*SDHA*

*SDHA*, located on 5p15, is a highly polymorphic gene encoding the flavoprotein subunit of SDH (figure 7)[136]. Until recently, no association between this major catalytic subunit of SDH and paraganglioma formation could be established. Instead, homozygous recessive SDHA mutations were associated with Leigh syndrome, a rare mitochondrial deficiency resulting in encephalopathy, myopathy, developmental retardation, loss of vision, loss of hearing, and a limited life expectancy[137]. In 2010, a heterozygous *SDHA* germ line mutation was identified in a single patient with a functional extra-adrenal paraganglioma of the abdomen. Loss of the wild type *SDHA* allele was found in the tumor tissue of this patient, suggesting that SDHA can act as a tumor suppressor in paragangliomas too[138].

The prevalence of SDHA mutations in the head and neck paraganglioma population remains to be clarified. Its late identification as a tumor suppressor and the isolated presentation of the reported SDHA-linked patient indicate that SDHA mutations are a rare cause of paraganglioma susceptibility[138]. The reasons for this infrequent association of SDHA mutations with paragangliomas are currently unknown. One explanation might be that mutations that eliminate all SDHA activity are incompatible with life. It has been shown that both the homozygous *SDHA* mutations causing Leigh syndrome as well as the heterozygous *SDHA* mutation causing paraganglioma result in SDH deficiency (see paragraph 4.1: 'succinate dehydrogenase')[137-139]. However, in patients with Leigh syndrome, considerable residual cytoplasmic SDHA immunostaining and activity can still be detected, indicating that SDHA stability is affected but SDHA functionality is not completely lost[137]. There is evidence that SDHA has a cellular function additional to its enzymatic role in the TCA cycle, as a component of the mitochondrial ATP-sensitive potassium channel[140,141]. Possibly, mutations that interfere with this other function are not tolerated.

An alternative explanation for the scarcity of SDHA-linked paragangliomas is a low observed frequency of somatic 5p15 loss[138,142]. Assuming that *SDHA* acts as a tumor suppressor gene in paragangliomas, loss of heterozygosity (LOH) targeting the wild type *SDHA* allele on 5p15 is an essential step in SDHA-linked tumorigenesis. The LOH at 5p15 may be prevented by local epigenetic factors or the LOH may simultaneously affect other genes in the close proximity of *SDHA* that are vital to the survival of the cell[138,140].

### 3.3 Other genes in paragangliomas and pheochromocytomas

Recently, germ line mutations in other genes that are not directly linked to SDH have also been identified in paraganglioma and pheochromocytoma patients. Although to date they do not seem to be very prevalent in the head and neck paraganglioma population, these discoveries may contribute to our insight in the tumorigenic pathways that are implicated in paraganglioma tumorigenesis (see: 'molecular biology of paragangliomas'), and are therefore discussed briefly.

*TMEM127*

In 2010, mutations in a three-spanner transmembrane protein, transmembrane protein 127 (TMEM127), have been identified as a cause of familial and isolated pheochromocytomas, and recently also of extra-adrenal paragangliomas and paragangliomas of the head and neck[143,144]. Mutations in *TMEM127*, located on 2q11, cause hereditary paraganglioma-pheochromocytoma syndrome with autosomal dominant inheritance, and LOH of the wild type *TMEM127* allele is observed in tumors indicating that *TMEM127* acts as a tumor suppressor gene (table 2)[143]. The function of TMEM127 is currently not fully known, but initial insights suggest that TMEM127 is a negative regulator of the mechanistic target of rapamycin (mTOR) pathway (see paragraph 4.4: 'other mechanisms in paraganglioma tumorigenesis')[143]. Clinically, TMEM127-linked patients are characterized by the occurrence of pheochromocytomas, which are frequently bilateral (in 35-50%), a comparative late onset of disease (mean age at diagnosis 42.8-45.3 years), and a low malignancy rate (0-5%)[143,145]. The penetrance of *TMEM127* mutations awaits detailed investigation, the first data suggest it is high but incomplete (an age related penetrance of 64% by the age of 43-55 years)[143-145]. The prevalence of *TMEM127* mutations in the paraganglioma and pheochromocytoma population seems low, recently a total of in 20 patients carrying 19 different mutations could be identified in an international cohort of 990 paraganglioma-pheochromocytoma patients (2%)[145].

*PHD2*

In 2008, a mutation in *EGLN1*, located on 1q42, was reported to be associated with an abdominal extra-adrenal paraganglioma in a single isolated patient (table 2)[146]. *EGLN1* encodes HIF-prolyl hydroxylase 2 (PHD2), an enzyme involved in the degradation of hypoxia inducible factor 1 alpha (HIF-1α). PHD2 mutations have also been associated with erythrocytosis, a rare neoplastic disorder causing an elevated red blood cell count, and the patient who developed the recurrent functional paraganglioma suffered from this condition too[146,147]. LOH of the wild type *EGLN1* allele was observed in the tumor, which indicates that PHD2 may act as a tumor suppressor in paraganglioma tumorigenesis[146]. The disruption of PHD2 function caused by this PHD2 mutation could

result in tumorigenisis through a pathway similar to that of defective SDH (see paragraph 4.2.3: 'pseudo-hypoxic drive' and figure 8)[146].

*KIF1B*

Also in 2008, kinesin family member 1B (*KIF1B*), located on 1p36, was identified as the causative tumor suppressor gene in a cancer-prone family suffering from multiple tumors, including neuroblastomas, ganglioneuromas, leiomyosarcomas, and pheochromocytomas[148]. It was found to act in a pro-apoptotic pathway downstream of prolyl-hydroxylase 3 (PHD3), a pathway that is involved in the development of neuronal precursor cells. Mutations in KIF1B could protect from apoptosis, causing aberrant survival of neuronal precursor cells that may later give rise to pheochromocytomas (see paragraph 4.3: 'abnormal development of neuronal precursor cells', and figure 9)[148-150]. Transcription analysis suggests that *KIF1B*-linked pheochromocytomas are more closely related to *NF1*- and *RET*-, than to *VHL*- and SDH-associated tumors[149].

*MAX*

In 2011, exome sequencing identified mutations in the MYC associated factor X gene (*MAX*) in hereditary pheochromocytoma patients that had tested negative for mutations in *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *NF1*, *RET*, and *VHL*[151]. In all, 12 patients carrying 8 different *MAX* mutations have been described, with an early mean age at diagnosis of 32 years, and high rates of bilateral pheochromocytomas (67%) and malignancy (25%), although these characteristics might in part reflect the selection criteria of the study[151]. As of yet, no MAX mutations have been reported in head and neck paragangliomas.

*MAX*, located on 14q23, behaves as a tumor suppressor gene[151]. MAX is a member of the basic helix-loop-helix leucine zipper (bHLHZip) family, which also includes myelocytomatosis viral oncogene homolog (MYC) and MAX dimerization protein 1 (MXD1). This family of transcription factors regulates cell proliferation, differentiation and apoptosis, and is linked with the mTOR pathway, the pathway to which TMEM127 is linked as well[151,152]. Mutations in MAX could lead to pheochromocytoma tumorigenesis through these proliferative pathways or through interference with apoptosis and neuronal development via the same pathway as KIF1B (see 4.3: 'abnormal development of neuronal precursor cells', paragraph 4.4: 'other mechanisms in paraganglioma tumorigenesis', and figure 9)[151,153,154].

**Table 2.** Summary of genes currently associated with paragangliomas and pheochromocytomas.

| gene | locus | protein function | inheritance | associated syndrome | paraganglioma predilection site |
|---|---|---|---|---|---|
| *NF1* | 17q11 | neurofibromin, RAS pathway regulator | autosomal dominant | neurofibromatosis type 1 (NF1) | adrenal |
| *RET* | 10q11 | receptor tyrosine kinase | autosomal dominant | multiple endocrine neoplasia (MEN 2a/2b) | adrenal |
| *VHL* | 3p25 | part of HIF degradation complex | autosomal dominant* | Von Hippel-Lindau syndrome (VHL) | adrenal |
| *SDHA* | 5p15 | flavoprotein, catalytic subunit of SDH | autosomal recessive** | Leigh syndrome, myopathy, encephalopathy | extra-adrenal |
| *SDHAF2* | 11q13 | assembly factor of SDH | autosomal dominant, parent-of-origin dependent | paraganlioma syndrome (PGL2) | head and neck |
| *SDHB* | 1p35-36 | iron-sulphur catalytic subunit of SDH | autosomal dominant | paraganlioma syndrome (PGL4) | extra-adrenal, adrenal |
| *SDHC* | 1q23 | anchoring subunit of SDH | autosomal dominant | paraganlioma syndrome (PGL3) | head and neck |
| *SDHD* | 11q23 | anchoring subunit of SDH | autosomal dominant, parent-of-origin dependent | paraganlioma syndrome (PGL1) | head and neck |
| *TMEM127* | 2q11 | mTOR pathway regulator | autosomal dominant | paraganlioma-pheochromocytoma | extra-adrenal, adrenal |
| *EGLN1* | 11q23 | prolyl-hydroxylase 2, HIF degradation | autosomal dominant | familial erythrocytosis | extra-adrenal |
| *KIF1B* | 1p36 | developmental culling, apoptosis | autosomal dominant | neural crest-derived tumor syndrome | adrenal |
| *MAX* | 14q23 | member of bHLHZip family of transcription factors | autosomal dominant | hereditary pheochromocytoma | adrenal |

*Von Hippel-Lindau (VHL) syndrome is caused by heterozygous VHL mutations and inherited in an autosomal dominant way. Homozygous recessive VHL mutations are found in Chuvash syndrome, a rare form of hereditary polycythemia. **Inheritance of the reported SDHA-linked paraganglioma appears to be autosomal dominant, whereas homozygous recessive SDHA mutations may cause Leigh syndrome.

Considerable differences in the proportion of hereditary cases and relative mutation frequencies have been reported in different patient cohorts and different parts of the world. In recent studies that have evaluated patients with at least one head and neck paraganglioma, approximately 18-33% are reported to present with a positive family history, and pathogenic gene mutations can be identified in 31-55% of the head and neck paraganglioma patients, most frequently in *SDHD* (19-79%) and *SDHB* (9-34%), and less frequently in *SDHC* (0-14%), *VHL* (0-2%) or *RET* (0-0.1%)[17,103,110,155,156].

In the Netherlands, the incidence of head and neck paraganglioma and the percentage of paraganglioma patients with a positive family history appear to be disproportionately high, due to the prevalence of Dutch founder mutations in SDH genes[118,119]. The relative frequency of SDH mutations in the Netherlands and the genetics of Dutch paraganglioma patients are further evaluated in chapters 3 and 4.

### 3.4 Inheritance of head and neck paraganglioma syndromes

The inheritance pattern of paraganglioma syndrome differs considerably depending on the causative gene involved (Table 2). While TMEM127-, SDHB- and SDHC-linked paraganglioma families show normal autosomal dominant inheritance, SDHD and SDHAF2-linked families show a virtually exclusive paternal transmission of tumor susceptibility[115,116,132,133]. Whereas mutations in SDHD and SDHAF2 can be inherited both via the maternal and paternal lines, tumor formation following maternal transmission of a mutation is exceedingly uncommon[115-117]. The absence of maternal transmission of disease in SDHD-linked paraganglioma families is suggestive of maternal imprinting of the *SDHD* gene[115]. However, the actual blocking of transcription by methylation of the *SDHD* gene itself has never been demonstrated, and *SDHD* shows bi-allelic expression in non-paraganglioma tissue[114,157,158]. Recently, it has been hypothesized that tissue specific hypermethylation of a maternal allele flanking the SDHD gene causes the imprinted inheritance of SDHD-linked disease. This flanking element is presumed to encode an alternative promotor of a non-coding RNA sequence in the vicinity of the SDHD promotor on 11q23[158]. The function of this non-coding RNA sequence however is unknown, and evidence of a regulatory role in *SDHD* expression is lacking. Although differential methylation of its putative alternative promotor was found, differential expression of the non-coding RNA sequence, the predicted result of differential methylation of its alternative promotor, could not be established in the majority of cases (86%). Moreover, no allelic imbalance was found for *SDHD*. In contrast, the report identified bi-allelic expression of *SDHD* in all non-paraganglioma tissues including the adrenal gland, in accordance with previous reports, and it is therefore highly unlikely that the reported differential hypermethylation in the vicinity of *SDHD* actually

affects *SDHD* expression[115,158]. Furthermore, differential hypermethylation was not found in SDHD-linked paragangliomas, and it consequently does not seem to play a role in the inheritance of SDHD-linked disease. An additional argument against hypotheses involving maternal imprinting of *SDHD* itself, or a selective reduction of the expression of the maternal *SDHD* allele due to other epigenetic factors, is that it does not explain the loss of the wild type maternal *SDHD* allele that is observed in SDHD-linked paragangliomas and pheochromocytomas[114,158-160]. If the expression of the wild type allele was already significantly reduced by imprinting phenomena, its loss would not confer an increased predisposition to tumorigenesis. Furthermore, these models do not explain the similar exclusive paternal transmission of disease observed in SDHAF2-linked cases[132,133].

A decisive factor in the parent-of-origin-dependent inheritance of SDHD- and SDHAF2-linked disease seems to be the location of *SDHD* and *SDHAF2* on chromosome 11. Both *SDHD* and *SDHAF2* are located on the long arm of chromosome 11 (on 11q23 and 11q13 respectively), while *TMEM127* (2q11), *SDHB* (1p35-36) and *SDHC* (1q23), genes that do not show a parent-of-origin effect, are not.

Although the *SDHD* and *SDHAF2* genes are not imprinted themselves, chromosome 11 harbors the main cluster of imprinted genes of the human genome, on its short arm at 11p15.5. This region consists of a telomeric and a centromeric imprinted domain, both containing putative tumor suppressor genes. This suggests a model in which a maternally expressed, paternally imprinted gene, located within this imprinted 11p15.5 region, is an essential initiator or modifier of tumor development in SDHD- and SDHAF2-linked paraganglioma syndromes[114]. According to this model, tumor formation is not initiated upon loss of the wild type *SDHAF2* or *SDHD* allele alone, but only upon the combined loss of the wild type *SDHAF2* or *SDHD* allele on the long arm of chromosome 11 and the active maternal tumor suppressor allele located within the imprinted 11p15.5 region. In case of a paternally inherited mutation in *SDHAF2* or *SDHD*, this can be achieved in a single event, i.e. the somatic loss of the whole maternal copy of chromosome 11 (figure 6). The infrequent maternal transmission of tumor susceptibly in SDHAF2 and SDHD-linked families would then be explained by the fact that it takes at least two separate events to eliminate both the paternal wild type *SDHAF2* or *SDHD* allele on the long arm of chromosome 11 and the active maternal copy of a paternally imprinted tumor suppressor gene on the 11p15.5 region (figure 6)[114]. In support of this model, multiple studies have found evidence for LOH targeting the 11p15 region and for the selective loss of maternal chromosome 11 alleles in SDHD-linked paragangliomas and pheochromocytomas[114,158,159,161].

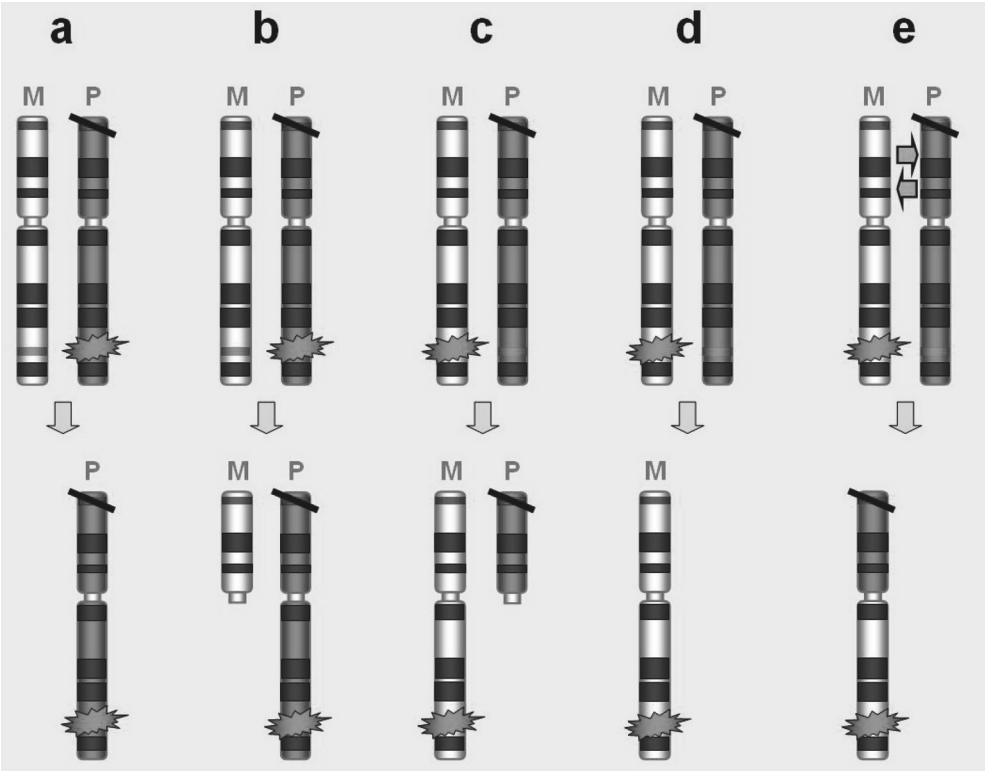



**Figure 6.** Model for the parent-of-origin-dependent transmission of SDHD-linked paraganglioma syndrome. The model is applicable to SDHAF2-linked disease as well. Maternal (M) and paternal (P) chromosome 11 copies are represented with the wild type (green band) and mutant (green star) *SDHD* alleles located on the long arm (11q23), and the active allele (red band) and imprinted allele (red band struck through) of a tumor suppressor gene on the short arm (11p15.5). (**a**) *SDHD* mutation inherited via the father. Loss of the whole maternal chromosome 11 copy targets both the wild type *SDHD* allele and the maternal 11p region containing the active tumor suppressor allele in a single event, resulting in tumor formation. (**b**) *SDHD* mutation inherited via the father. In case of loss of heterozygosity (LOH) targeting only the wild type maternal *SDHD* allele on 11q, the active maternal tumor suppressor allele on 11p15.5 is not affected and tumor development is inhibited. In case of maternal inheritance of the *SDHD* mutation, a second hit targeting the wild type paternal allele by, for example, a deletion of the paternal 11q region (**c**) or even the whole paternal chromosome 11 (**d**) will leave the maternal 11p15.5 region intact and tumor formation is not initiated. When the *SDHD* mutation is maternally transmitted, at least two separate events are required to inactivate both the wild type *SDHD* allele and the active allele of the imprinted tumor suppressor gene on 11p15.5. To date, true maternal transmission of SDHD-linked disease has been found in association with loss or disruption targeting both the wild type paternal *SDHD* allele and the maternal chromosome 11p15.5 region, either by two separate LOH events (reported by Tobias et al.), or by an altered imprinting status at 11p15.5 in combination with loss of the wild type paternal *SDHD* allele (described by Pigny et al.), or (**e**) through a recombination on chromosome 11 followed by loss of the wild type paternal *SDHD* allele and maternal 11p15.5 region (observed by Bayley et al.). Apparently, these sequences of events occur very rarely in vivo.

To date, very few cases of true maternal transmission of paragangliomas associated with *SDHD* mutations have been reported. Pigny et al. have described a patient that developed a tympanic paraganglioma after inheriting a *SDHD* mutation via his mother. Unlike his unaffected family members, he had also acquired an altered methylation profile and therefore probably an altered imprinted status of *H19*, a known paternally imprinted tumor suppressor on 11p15.5[117,162]. However, in this case the diagnosis of paraganglioma was not confirmed by histopathology, and loss of the paternal *SDHD* allele or alterations in H19 expression could not be evaluated in the tumor tissue[114,117,163]. The second report of maternal transmission identified a pheochromocytoma in a maternally derived SDHD mutation carrier, after two separate LOH events had resulted in loss of the paternal wild type SDHD allele and loss of the maternal 11p15.5 region in the tumor[164]. A third case of maternal transmission of SDHD-linked disease was observed by Bayley and co-workers (presented in 2011 by Bayley et al. at the International Symposium of Phaeochromocytoma and Paraganglioma, Paris, France). In this case, it was demonstrated that an entire copy of chromosome 11 was lost after somatic recombination on chromosome 11, resulting in loss of the paternal wild type *SDHD* allele and the maternal 11p15.5 region in the patients' pheochromocytoma (figure 6). These rare observations, or 'exceptions to the rule', are all consistent with the proposed model, and point to the maternal 11p15.5 region as an essential additional factor in paraganglioma formation. The model explaining the parent-of-origin dependent inheritance of SDHD- and SDHAF2-linked paraganglioma syndrome is presented in chapter 7.

Altered expression of 11p15.5 imprinted genes, especially *H19* and insulin-like growth factor 2 (*IGF2*), has been linked to other tumors and tumor syndromes, such as the Beckwith-Wiedemann syndrome (BWS), a pediatric developmental disease characterized by overgrowth, organomegaly, and a predisposition for various benign and malignant tumors, including, interestingly, pheochromocytomas[165,166]. LOH of 11p15.5 region is also observed in nephroblastoma, embryonal rhabdomyosarcoma, hepatoblastoma and adrenal cortical carcinoma, and in these tumors too, it has been shown that the LOH specifically targets maternal alleles[167]. In VHL-related pheochromocytomas, loss of maternal 11p15.5 alleles is also frequently observed, suggesting that the maternal 11p15.5 region has an important role in the tumorigenesis of paraganglioma-pheochromocytoma syndromes irrespective of the causative gene[168-170]. Moreover, the parent-of-origin-dependent inheritance described in focal hyperplasia of Langerhans islets causing congenital hyperinsulinism (FoCHI), a disease caused by genes that like *SDHD* and *SDHAF2* are located outside of the imprinted region on chromosome 11, and its association with altered expression of 11p15.5 imprinted genes, suggest that this model for parent-of-origin dependent inheritance of disease susceptibility may have implications beyond the spectrum of paraganglioma-pheochromocytoma syndromes[171].

# 4. Molecular biology of paragangliomas

In recent years, great progress has been made in elucidating the processes that lead from gene mutations to paraganglioma formation. Especially since the discovery of the SDH genes in hereditary paraganglioma syndrome, the understanding of the role of the cellular metabolism and hypoxia in tumor formation has evolved. The genes that cause paraganglioma formation, and the processes they regulate, are diverse and several pathways may be implicated in paraganglioma tumorigenesis. In this paragraph, the current models and insights in the tumor biology of paragangliomas are discussed. These models are not mutually exclusive, probably multiple mechanisms interact, and the relative role of each of these mechanisms in paraganglioma formation is not yet defined.

### 4.1 Succinate dehydrogenase

Most genes currently known to cause hereditary paraganglioma syndrome, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, and the recently implicated *SDHA* gene, encode subunits or co-factors of succinate dehydrogenase (SDH). SDH is an enzyme anchored to the inner membrane of mitochondria, which couples the oxidation of succinate to fumarate in the tricarboxylic acid cycle (TCA cycle, also known as Krebs cycle), with the transfer of electrons as the complex II component of the electron transport chain. It thus connects the TCA cycle with the mitochondrial respiratory chain, which places SDH at the center of two of the essential energy producing processes of the cell. SDH consists of a catalytic domain formed by the SDHA flavoprotein, which is involved in succinate binding and oxidation, the SDHB iron-sulfur protein, which is involved in the electron transfer, and a membrane-anchoring domain formed by the hydrophobic SDHC and SDHD subunits that also play a role in passing electrons through the electron transport chain (figure 7). *SDHAF2* encodes a protein that is involved in the incorporation of the flavin-adenine-dinucleotide (FAD) group into the SDHA subunit.

It has been demonstrated that mutations in each of these subunits or co-factors result in compromise of enzymatic function of the SDH complex[132,138,172-174]. The loss of SDH function is thought to interfere both with the TCA cycle as well as the mitochondrial respiratory chain, and it is therefore not readily apparent how defects in SDH can initiate such an energy draining process as tumor formation (see paragraph 4.2: ‘the Warburg hypothesis’)[175].

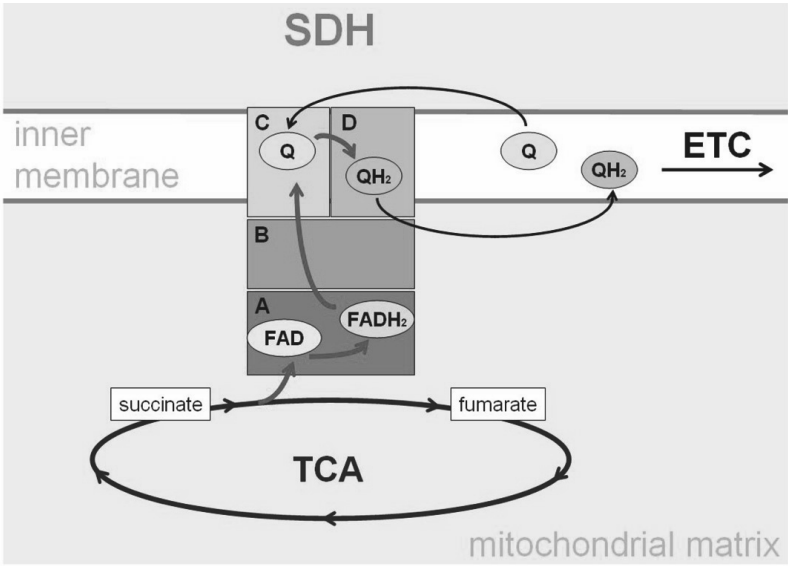



**Figure 7.** Schematic representation of the succinate dehydrogenase (SDH) complex, or mitochondrial complex II, and its dual role in the tricarboxylic acid cycle (TCA, blue circle) and the electron transport chain (ETC, black arrow). SDH consists of four subunits, SDHA (A), SDHB (B), SDHC (C) and SDHD (D). SDHA and SDHB form its catalytic domain, while SDHC and SDHD anchor the complex to the inner membrane of the mitochondrion. The electron flow within complex II is depicted by the red arrows. During the oxidation of succinate to fumarate by SDHA, its FAD group is reduced to FADH2 by two electrons. The electrons are then transferred through the iron–sulphur groups in SDHB to SDHC and SDHD, where ubiquinone (Q), bound to the mitochondrial inner membrane, is reduced to ubiquinol (QH2). Ubiquinol transfers its electrons to complex III, further in the electron transport chain (not shown).

### 4.2 The Warburg hypothesis

Already in 1926, the biochemist Otto Warburg postulated that cancer was caused by defects in the oxidative phosphorylation within mitochondria, after observing that cancer cells display high rates of glycolysis even in aerobic conditions, an effect he named 'aerobic glycolysis'. This metabolic shift, known as the 'Warburg effect', has since been recognized as a feature of many cancer types. The concept that disruptions in the mitochondrial respiration can actually cause cancer has long been controversial however (see paragraph 5.3: 'the Warburg controversy'). Today, the discovery of mutations in SDH subunits as a cause of paragangliomas and pheochromocytomas serves as a clear example of the tumorigenic potential of deficient TCA cycle components.

The molecular basis for the initiation of tumor growth by defective SDH has not been fully elucidated. Currently, there are three main models that link SDH disruption with

neoplastic growth: an increase in the formation of reactive oxygen species, a decrease in apoptosis or programmed cell death, and an activation of hypoxia pathway signaling under normoxic conditions (or 'pseudo-hypoxic drive')[175-177].

### 4.2.1 Reactive oxygen species

Reactive oxygen species (ROS) are highly reactive molecules containing oxygen. ROS are produced by the electron transport chain, predominantly at the site of complex I and complex III, as electrons are transferred to oxygen molecules. Although ROS production does not seem to take place at complex II (SDH) under physiological conditions, mutations in SDH subunits have been associated with increased levels of ROS[175,178,179].

It is currently unknown how mutations in SDH subunits lead to ROS production, but there are several hypotheses. First, mutant SDH might not conduct the electrons that are released in the process of the oxidation of succinate, resulting in a buildup of electrons at the FAD site of the SDHA subunit (figure 7). When sufficient electrons have accumulated, this site could overflow, leading to a direct transfer of electrons to oxygen, thus creating ROS[175,176,180]. Alternatively, the oxidation of succinate to fumarate may be reversed at the SDH complex under hypoxic conditions, generating ROS in the process[175]. Both hypotheses involve an active catalytic domain of SDH, whereas it has been shown that SDH mutations disrupt its enzyme activity[172,176]. A third hypothesis states that the SDH complex has a role in reducing the levels of ROS produced by other electron transfer chain components using its heme group, and that mutations in SDH interfere with this functionality[180,181].

The actions of ROS are diverse and in the light of tumorigenesis seemingly contradictory. On the one hand, ROS are known initiators of apoptosis[175]. On the other hand, the production of ROS can induce neoplastic growth by causing direct damage to the DNA, including strand breaks, cross-links and base modifications which may result in replication errors, altered gene expression, genomic amplification or LOH[175,182]. In addition, increased ROS production is associated with increased hypoxia pathway signaling and hypoxia-induced transcription, processes that have been shown to play a key role in paraganglioma tumorigenesis (see paragraph 4.2.3: 'pseudo-hypoxic drive')[173,183].

In paragangliomas, excess ROS production might be the result of defects in SDH caused by mutations in SDH genes and subsequent LOH of wild type SDH alleles. Alternatively, ROS production may already be increased in heterozygous SDH mutation carriers that have not yet lost the wild type SDH allele, and the high levels of ROS might drive the LOH of SDH genes. Whether or not heterozygous SDH mutations cause ROS excess is still a matter of

debate. Interestingly, heterozygous *Sdhd* mutant mice exhibit slight changes in carotid body physiology that may implicate increased levels of ROS (see paragraph 4.9: 'insights from mouse models')[43,184].

### 4.2.2 Evasion of apoptosis

In addition to their role in the energy supply of the cell, mitochondria have a central role in programmed cell death or apoptosis. Apoptosis may be induced by increasing the permeability of the mitochondrial membrane, resulting in the release of apoptogenic proteins such as cytochrome-c into the cytosol, or by interfering with the bioenergetic processes within the mitochondria. Evasion of apoptosis is one of the acquired hallmarks of neoplastic growth[185]. Several mechanisms may cause insensitivity to apoptotic signals in paragangliomas. First, in SDH mutant paragangliomas, SDH deficiency causes the oxidative phosphorylation to operate at suboptimal levels. This causes an induction of glycolysis as the alternative energy producing pathway, through upregulation of glycolytic enzymes such as hexokinase and glycerylaldehyde-3-posphatase (GAPD). Hexokinase and GAPD are proteins that are implicated in the regulation of diverse other cellular processes, including apoptosis, and the induction of glycolysis could thus have an anti-apoptotic effect[175,186]. Alternatively, SDH deficiency causes an accumulation of succinate (see paragraph 4.2.3: 'the hypoxia pathway'), which in turn can inhibit the pro-apoptotic activity of prolyl-hydroxylase 3 (PHD3) (see paragraph 4.3: 'abnormal development of neuronal precursor cells')[150,176,187].

A third link between SDH and apoptosis that has been put forward, is a decrease rather than an increase in ROS production under hypoxic conditions due to defects in SDH, which would interfere with the pro-apoptotic signal transduction by ROS in mitochondria[188;189].

A fourth mechanism involves the B-cell lymphoma 2 (BCL-2) family of apoptosis regulating proteins. Two of its members, BCL-2 and BCL-xl, have been shown to counteract the pro-apoptotic signaling by ROS and hypoxia, and prevent the release of cytochrome-c from the mitochondrial membrane[190-192]. The upregulation of BCL-2 and BCL-xl is a known response to hypoxia and has been observed in paragangliomas[193-195]. Another member of the BCL-2 family, BCL-2 interacting protein 3 (BNIP3), acts as a promoter of apoptosis, and has been shown to be repressed in certain SDHB-linked tumors[196].

There are several observations that implicate blocked apoptosis in SDH-linked paraganglioma tumorigenesis. Douwes Dekker et al. found very few morphological stages of apoptosis and no DNA strand breaks suggesting a reduced apoptotic activity in paragangliomas[193]. In addition, it has been shown that whereas short periods of

SDH deficiency can induce apoptosis, prolonged SDH deficiency can result in an absent apoptotic response and induction of tumorigenesis[178,179]. Further evidence for the role of SDH in apoptosis comes from the study of new possible anti-cancer agents such as vitamin E analogs, that are able to induce apoptosis only in the presence of functional SDH and not in SDH deficient cells[197].

### 4.2.3 The hypoxia pathway

Long before the identification of any of the genes now known to play a role in paragangliomas, it was recognized that living at high altitude increases the risk of carotid body hyperplasia and carotid body tumors[198,199]. In addition, carotid body hyperplasia also occurs in patients suffering from cystic fibrosis or cyanotic heart disease, conditions associated with compromised gas exchange in the lungs[200]. This increased prevalence of paragangliomas in conditions characterized by low oxygen levels and the central role of the carotid body in oxygen sensing suggested that hypoxia or defects in the oxygen sensing mechanism play a role in the tumorigenesis of paragangliomas.

*Oxygen sensing at the carotid body*

Corneille J.F. Heymans was the first to demonstrate the role of the carotid body as a peripheral arterial chemoreceptor and regulator of respiration and oxygen homeostasis (see paragraph 5.2: 'the discovery of the carotid body function')[5]. Oxygen sensing takes place in the type 1 or chief cells, primarily within the carotid body[201]. Type 1 cells are polymodal chemoreceptors that are sensitive not only to low oxygen, but also to carbon dioxide, extracellular pH, and glucose levels, however the oxygen sensing ability is what makes the carotid body essential in the adaptive hyperventilatory reflex response[200]. Hypoxia elicits the release of two classes of neurotransmitters by the chief cells: conventional neurotransmitters such as acetylcholine, catecholamines, substance P and adenosine triposphate (ATP), and unconventional neurotransmitters such as nitric oxide (NO) and carbon monoxide (CO)[43,202]. Acetylcholine and ATP in particular seem to be responsible for the excitation of afferent endings of the carotid sinus nerve and to increase impulse traffic to the brain stem, thus regulating the hypoxic ventilatory response[43,200,202].

*Acute hypoxia response*

There are two main models for carotid body oxygen sensing: the 'membrane model', designating potassium channels as the initiators of the response to hypoxia, and the 'mitochondrial model', which involves heme containing proteins like nitric oxide synthetase (NOS), hemoxygenase 2 (HO-2), NADPH oxidase, and/or the mitochondrial complexes such as SDH as the main hypoxia responsive elements[43,202]. Thus far, the identification of

one specific compound as the central oxygen sensor remains elusive. Several interacting molecular mechanisms are believed to be involved but the full complexity of the process is not yet entirely understood.

Central to the membrane model of oxygen sensing is the concept that hypoxia induces an altered conductance of potassium channels located in the membrane of type 1 cells, which causes depolarization and the influx of calcium in the cytosol, triggering the release of neurotransmitters[43,200,202]. There are several pathways that might link hypoxia to potassium channel function and probably different types of potassium channels are involved. It has been hypothesized that low oxygen levels could directly alter the open probability of potassium or calcium channels in type 1 cells, causing depolarization and transmitter release[43,201]. Alternatively, potassium channels can be affected by increased levels of ROS or decreased levels of ATP as a result of hypoxia (see below)[202].

In the mitochondrial model, heme containing compounds such as NOS, HO-2, NADPH oxidase, and/or the mitochondrial complexes such as SDH are viewed as the primary starting-point of the ventilatory response to hypoxia. This response may be regulated by NOS through the production of nitric oxide (NO), which may play a role in suppressing sensory discharge in type I cells under normoxic conditions, and may have modulating effects on hypoxia induced neurotransmitters[201]. A reduced activity of NOS results in low levels of NO, relieving its inhibitory effect on type 1 cells[201]. Whether or not NOS activity and NO levels are actually altered by hypoxia within type 1 cells is yet unclear, but NO produced in nearby nerve terminals might also exert this modulating effect[43,202].

Hemoxygenase 2 could be involved in oxygen sensing through the regulation of carbon monoxide (CO) levels. Hemoxygenase 2 is capable of endogenous production of CO in type 1 cells, in a process that is oxygen dependent. Endogenous CO is thought to exert an inhibitory influence on carotid body function, but the effects of CO on oxygen sensing seem to be of a dual nature: high concentrations of CO inhibit NOS activity and augment sensory discharge in type 1 cells (see above)[201-203].

The mechanism linking the non-mitochondrial enzyme NADPH oxidase or the mitochondrial electron transport chain complexes, including SDH, to oxygen sensing in the carotid body involves the production of ROS by these enzymes as a function of the amount of oxygen available to the cell. Low concentrations of oxygen result in decreased levels of ROS, which would in turn alter the open probability of the potassium channels[43,201]. In addition, mitochondrial complexes could alter the conductance of ATP-dependent potassium

channels, causing depolarization and transmitter release, through a reduced production of ATP in response to low oxygen levels[43,201].

*Chronic hypoxia*

Chronic hypoxia, i.e. an exposure to hypoxic conditions lasting several hours or longer, induces a number of morphological, electrochemical and physiological adaptations that increase the responsiveness of oxygen sensing mechanisms, promote oxygen delivery to tissues, and adjust the cellular metabolism to limited oxygen availability[43,202]. Most of these effects are regulated through hypoxia inducible factor 1 (HIF-1). HIF-1 is a heterodimeric transcription factor composed of the HIF-1α and HIF-1β subunits. HIF-1β is constitutively expressed, whereas HIF-1α levels increase exponentially as oxygen levels decrease. Under normoxic conditions, HIF-1α levels are reduced, primarily by the activity of prolyl-hydroxylases (PHDs) 1, 2 and 3, which modify HIF-1α so that it can be ubiquitinated by a complex consisting of the Von Hippel-Lindau protein (pVHL) and an E3 ubiquitin-protein ligase, after which it is targeted for proteasomal degradation. Under hypoxic conditions, HIF-1α degradation by PHDs is inhibited either by deprivation of oxygen (a substrate of the PHDs), or as a result of oxidation of the iron group within the PHDs by ROS, resulting in accumulation of HIF-1α. Subsequently, HIF-1α translocates to the nucleus and dimerizes with the HIF-1β subunit, forming the HIF-1 transcription factor[204,205]. HIF-1 binds to hypoxia response elements (HREs) and activates the transcription of a large number of genes that are involved in cell proliferation, survival, apoptosis, glucose transport and metabolism, angiogenesis, cytoskeletal structure and motility, and extracellular matrix metabolism (see also chapter 6)[205-207].

Two other isoforms of HIF-1α exist: HIF-2α and HIF-3α. HIF-2α is a protein with extensive similarity to HIF-1α, its degradation is also regulated in an oxygen-dependent way by PHDs, it also dimerizes with HIF-1β, and it regulates the transcription of an overlapping, but not identical set of genes. HIF-3α is transcriptionally regulated by HIF-1 and acts as an inhibitor of HIF-1[205,206].

*The tumorigenic effects of hypoxia*

Both HIF-1α and HIF-2α overexpression is associated with the development and behavior of a large variety of neoplasms. It has been shown to increase resistance to chemotherapy, radiotherapy and photodynamic therapy, and to promote tumor growth, vascularization, metastasis, and mortality in melanomas, oligodendromas, astrocytomas, and multiple types of carcinoma[183,206]. The effects of HIF-1α and HIF-2α overexpression are not universal however, and the biological consequences of HIF-1 activation depend on the specific subset of genes that responds[183,206]. The HIF-1 activation can be induced by

intratumoral hypoxia in areas distal to blood vessels and on the border of necrotic cells, or by genetic alterations to hypoxia pathway components (see paragraph 4.2.3: 'pseudo hypoxic drive')[183,205].

*Pseudo-hypoxic drive*

In some tumors, the tumorigenic effects of hypoxia are induced under normoxic conditions, an effect known as the 'pseudo-hypoxic drive'. This pseudo-hypoxic drive can be caused by genetic mutations that affect pathways regulating the transcriptional activity HIF-1 in an oxygen-independent way[204]. In SDH-linked tumors, there are several possible routes linking the activation of HIF-1 with SDH deficiency (figure 8). The first involves the production of ROS as a result of SDH disruption (see paragraph 4.2.1: 'reactive oxygen species'). When sufficient amounts of ROS accumulate, ROS can oxidize the iron group within PHDs, thereby decreasing PHD activity and increasing HIF-1α (and HIF-2α) stability. As explained above, blocking of HIF-1α degradation will lead to increased HIF-1 mediated transcription and activation of the hypoxia pathway (figure 8)[175]. In addition, there is evidence that ROS can increase HIF-1 stability directly (figure 8)[208]. The second route is through the accumulation of succinate, the substrate of SDH in the TCA cycle. In the cytosol, succinate is also present as a product of the conversion of α-ketoglutarate by PHDs. The accumulation of succinate in the mitochondrion as a result of SDH deficiency, and the subsequent transport of excess succinate to the cytosol, leads to high levels of cytosolic succinate, which prevents the forward hydroxylation of HIF-1α by PHDs, resulting in increased HIF-1 activity (figure 8)[175,209,210].

An alternative pathway linking SDH deficiency with HIF-1 activity is through the reduced activity of other hydroxylases, such as the factor inhibiting HIF (FIH). FIH reduces HIF-1 mediated transcription by preventing the recruitment of co-activators[205,211,212]. Hydroxylation of HIF by FIH, like hydroxylation of HIF by PHDs, requires the co-factors iron and oxygen and α-ketoglutarate as a co-substrate, and FIH activity can be blocked by ROS and high levels of succinate in the same way as PHD activity (see above), resulting in increased HIF-1 transcription[212].

There is ample evidence implicating the hypoxia pathway in paraganglioma tumorigenesis. First, HIF-1α and HIF-2α stabilization, the accumulation of succinate, and its inhibitory effect on PHDs have all been demonstrated in SDH deficient cells and paragangliomas[209,210]. Second, the fact that paragangliomas and pheochromocytomas can be caused by mutations in SDH subunits as well as by mutations in VHL and PHD2, points towards the hypoxia pathway in paraganglioma tumorigenesis because of the role that SDH, VHL and PHD2 all have in the stabilization of HIF-1α and HIF-2α[213]. Third, HIF-1

regulated genes have been shown to be overexpressed in SDH-linked paragangliomas and pheochromocytomas, and this is a very plausible explanation for some of the clinical characteristics of paragangliomas, such as their typical high vascularity (see paragraph 4.4: 'angiogenesis')[173,213].

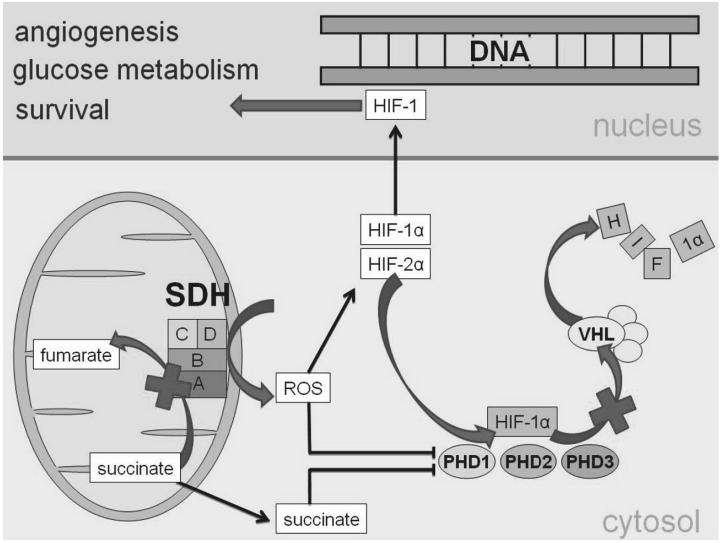



**Figure 8.** Schematic representation of the pseudo-hypoxic drive. Defects in succinate dehydrogenase (SDH), prolyl-hydroxylase 2 (PHD2) and the Von Hippel-Lindau protein (pVHL) all inhibit the degradation of hypoxia-inducible factors HIF-1α and HIF-2α. The HIF alpha subunits subsequently combine with HIF-1β to form the transcription factor HIF-1. HIF-1 activates the transcription of a multitude of genes involved in glucose metabolism, angiogenesis, survival, cell motility and extracellular matrix metabolism. The stabilization of HIF-1α and HIF-2α subunits can be induced in several hypoxia-independent ways: defects in SDH interfere with the oxidation of succinate to fumarate in the TCA cycle, resulting in accumulation of succinate. Succinate is transported to the cytosol, where it blocks the activity of PHD 1, 2, and 3 by product inhibition. Alternatively, defects in SDH result in the production of reactive oxygen species (ROS), which disable PHDs by oxidizing the iron group within, or may contribute to the stability of HIF-1α directly. Mutations in PHD2 can affect its ability to catalyze the hydroxylation of HIF-1α. Mutations in the Von Hippel-Lindau protein (pVHL) disrupt HIF-1α ubiquitination.

### 4.3 Abnormal development of neuronal precursor cells

An alternative hypothesis for the development of paragangliomas and pheochromocytomas explains their tumorigenesis not primarily by defects in the cell metabolism or pseudo-hypoxia, but through the faulty development of sympathetic neuronal precursor cells that give rise to the sympathetic nervous system as well as to the paraganglionic type 1 cells. During normal development, damaged or unneeded precursor cells originating from

the neural crest are disposed of by apoptosis in a process called 'developmental culling'. This process is regulated by growth factors, most notably nerve growth factor (NGF) through a pathway involving the c-Jun protein and prolyl-hydroxylase 3 (PHD3, encoded by *EGLN3*) (figure 9). Under normal circumstances, the precursor cells undergo apoptosis when NGF becomes limiting, but it has been demonstrated that pheochromocytoma cells might escape the developmental culling by blocking apoptosis through the c-Jun/PHD3 pathway[150]. This model for paraganglioma and pheochromocytoma development is attractive because the SDH genes, *NF1*, *RET*, *VHL*, *KIF1B* and *MAX* can all be linked to this essential mechanism in the development of the paraganglion system (figure 9)[149,151]. Moreover, there is some evidence from knockout mouse models that associates the developmental stages of the nervous system with pheochromocytoma formation (see paragraph 4.5: 'insights from mouse models'). However, the hypothesis implies that the second hit targeting the wild type allele of these tumor suppressor genes occurs very early in life, or that heterozygous mutations already exert an inhibitory effect on this pathway. In case of SDH mutations, there is currently no evidence to support that heterozygous SDH mutations result in succinate accumulation, and it is therefore uncertain whether heterozygous SDH mutations can induce aberrant survival of neuronal precursor cells (figure 9).

### 4.4 Other mechanisms in paraganglioma tumorigenesis

*Cell cycle arrest*

The process of the replication of cells, called the cell cycle, comprises of multiple phases: the G1- or growth phase, the S- or DNA replication phase, the G2 phase in which microtubules are formed, and finally, the M- or mitotic phase in which the actual division of nuclear DNA takes place. Each transition into the next phase is guarded by a checkpoint that is very tightly regulated through multiple complex mechanisms. In order to attain uncontrolled proliferation, a hallmark of cancer, the neoplastic cell must evade these checkpoints, and in most forms of cancer the mitotic rate, i.e. the proportion of replicating cells, is high. In paragangliomas however, a very low mitotic rate has been observed, and a large number of cells seem to be stranded in the G2 phase of the cell cycle, indicating that some cell cycle regulation is still operative[193]. The low mitotic rate might explain the indolent behavior and slow growth that characterizes most paragangliomas. Cell cycle arrest can be caused by hypoxia, through upregulation of tumor protein 53 (p53), one of the most well-known regulators of the cell cycle and apoptosis, which regulates the G2/M checkpoint through a complex cascade. Mutations in p53 confer a growth advantage as the cell is less able to respond to hypoxia or DNA damage with cell cycle arrest and apoptosis, and p53 mutations are implicated in a vast array of neoplasms, but are an infrequent finding in paragangliomas[193,214]. It is therefore conceivable that the cell cycle arrest

observed in paragangliomas is the result of the intact response of p53 to pseudo-hypoxia caused by SDH deficiency, however, p53 overexpression also is not a characteristic of most paragangliomas[193,214].

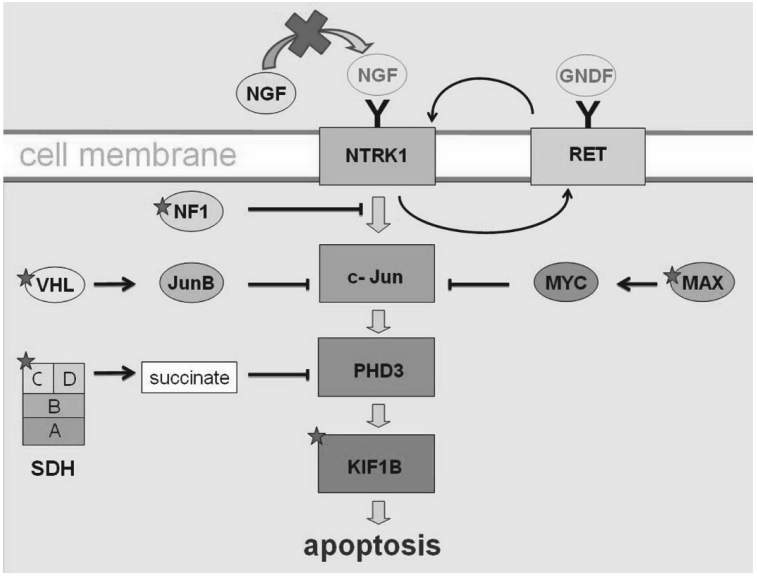



**Figure 9.** Almost all paraganglioma and pheochromocytoma genes converge on the pro-apoptotic pathway that is involved in the developmental culling of neuronal precursor cells. NTRK1, the receptor for nerve growth factor (NGF), collaborates with the RET tyrosine kinase receptor (RET) to regulate NGF and glial cell line- derived neurotrophic factor (GDNF) signals. Loss of NGF leads to apoptosis under normal conditions. Mutations (red stars) in neurofibromin 1 (NF1) interfere with downstream signaling by the NTRK1 receptor. Mutations in the Von Hippel-Lindau protein (VHL) cause induction of the JunB protein, which in turn antagonizes the pro-apoptotic activity of c-Jun. The upregulation of c-Jun can also be blocked by mutations in MYC associated factor X (MAX) through altered activity of myelocytomatosis viral oncogene homolog (MYC). The hydroxylation activity of prolyl-hydroxylase 3 (PHD3), which is transcriptionally activated by c-Jun, can be blocked through product inhibition by increased levels of cytosolic succinate. The accumulation of succinate can be caused by disrupted enzymatic activity of succinate dehydrogenase (SDH) due to mutations in the SDH subunits. Kinesin family member 1B (KIF1B) regulates apoptosis downstream from PHD3, and KIF1B mutations may therefore also result in blunting of the pro-apoptotic signal through this pathway.

An alternative pathway that links pseudo-hypoxia with cell cycle arrest is through the upregulation of cyclin-dependent kinase inhibitor 1 (CDKN1A or p21) by HIF-1. The p21 protein is regulated by p57, but is also a transcriptional target of HIF-1[207;215]. The p21 protein is capable of inducing cell cycle arrest in G1/S- and G2/M transitions, and has been shown to be expressed in paragangliomas[193,215]. Interestingly, prolonged G2/M

arrest through induction of p21 is also associated with polyploidisation, another feature frequently encountered in the nuclei of type 1 paraganglioma cells[114,193,215].

Whereas p21 and p57 have been studied in paragangliomas, many other pathways that regulate the cell cycle have yet to be investigated, and the exact mechanism of cell cycle arrest in paragangliomas remains to be elucidated[193,214].

*Angiogenesis*

Paragangliomas are characterized by a highly vascular stroma and close relations to adjacent vascular structures. Histologically, the 'Zellballen' clusters of type 1 and type 2 cells are surrounded by a very prominent capillary network[4,216]. The mechanism by which tumors induce the sprouting and development of blood vessels from existing vasculature, called angiogenesis, is an essential step in neoplastic growth and progression, as the expanding tumor requires increasing amounts of nutrients and oxygen to sustain itself[212,217]. One of the critical triggers of angiogenesis is hypoxia within a tumor, and virtually all of the central mediators in the process, such as vascular endothelial growth factor (VEGF), platelet derived growth factor b (PDGFB), stromal derived factor 1 (SDF1), angiopoietin 2 (ANGPT2), fibroblast growth factor 2 (FGF2), and important receptors are regulated through the hypoxia pathway by HIF-1 (see also chapter 6)[205-207,218]. Both the stabilization of HIF-1α and HIF-2α lead to the expression of angiogenic factors, but there is evidence that HIF-2α has a more prominent role in angiogenesis[212]. In paragangliomas, angiogenesis could be initiated by pseudo-hypoxia signaling (see paragraph 4.2.3: 'pseudo-hypoxic drive'). HIF-1α, HIF-2α and various HIF-1 target genes involved in angiogenesis such as FGF2, VEGF, and VEGF receptor 1, have indeed been shown to be upregulated in type 1 cells of paragangliomas and pheochromocytomas, indicating that the pseudo-hypoxic drive is responsible for the vascular nature of these tumors[196,219,220].

*Proliferative pathways*

The recent discovery of TMEM127 and MAX as a tumor suppressors in hereditary paraganglioma and pheochromocytoma syndromes, and their roles as regulators of the mTOR pathway and the MYC-MAX-MXD1 family of transcription factors respectively, indicate that HIF-independent proliferative pathways may also play a role in paraganglioma formation (see paragraph 3.3: 'other genes in paragangliomas and pheochromocytomas') [143,151]. Gene expression studies have indicated that pheochromocytomas may be classified into two broad categories: one consisting of VHL and SDH-related tumors, and another consisting of NF1 and RET-related tumors (see paragraphs 3.1 and 3.2)[213]. The VHL/SDH associated tumors are characterized by an expression signature of angiogenesis,

hypoxia, and a suppression of the mitochondrial oxidative response and TCA cycle components, consistent with their role in HIF-1 regulated transcription (see paragraph 4.2.3: 'pseudo-hypoxic drive'). The NF1/RET associated tumors are characterized by upregulation of a distinct set of biological programs, including translation, protein synthesis, and kinase signaling, in agreement with their respective gene functions (see paragraph 3.1: NF1, RET, and VHL) [213]. The transcriptional profile of TMEM127-related tumors shows similarities with the NF1/RET cluster, and mTOR signaling has been shown to be important in NF1 associated tumorigenesis as well[213,221]. However, whereas TMEM127-linked tumors are associated with the mTOR signaling pathway, NF1 and RET-linked tumors are more closely associated with the mitogen-activated protein kinase (MAPK) pathway[143,213]. MAX has been linked to both the mTOR and MAPK pathways through the MYC-MAX-MXN1 network[152,222].

The MAPK and mTOR pathways are highly complex, and regulate a multitude of cellular processes including transcription, cell proliferation and survival, and both pathways have been implicated in various forms of cancer[223,224]. Here, the MAPK and mTOR pathways will not be discussed in detail, but some interesting links to the hypoxia pathway exist. Like the hypoxia pathway, both MAPK and mTOR signaling can be induced by hypoxia and oxidative stress, both MAPK and mTOR signaling can be induced by growth factors that are known HIF-1 target genes, and both MAPK and mTOR pathways have a regulating effect on HIF-1 transcriptional activity (and vice versa), illustrating the intricacy and interdependence of the different pathways that may lead to paraganglioma tumorigenesis[205,218,223-225].

### 4.5 Insights from mouse models

Several studies have reported on the effects of inactivation of genes associated with paragangliomas and pheochromocytomas in genetically modified mice (knockout mouse models). Whereas paraganglioma development is extremely rare in mice, life-span studies of laboratory mouse strains report a risk of 0-5% for the development of pheochromocytomas, and a higher prevalence in several genetically engineered strains[226]. Mice are the most widely used species for the knockout technique, because they are the closest related animal species in which the technique can be applied with relative ease. Mouse models can be used to study the effect of specific gene mutations and evaluate determinants of tumor behavior. The available models for the paraganglioma and pheochromocytoma genes that have been discussed in former paragraphs will be briefly evaluated here.

*Sdhd*

Several studies have demonstrated that homozygous disruptions of the *Sdhd* gene (*Sdhd* -/-) in mice invariably result in mortality early in embryogenesis[184,227,228]. In heterozygous *Sdhd* knockout mice (*Sdhd* +/-), the response of the carotid body to acute hypoxia remains largely intact, although a higher rest-excitability and basal catecholamine release has been demonstrated, probably due to potassium channel dysfunction and persistent calcium influx (see paragraph 4.2.3: 'oxygen sensing at the carotid body') [43,184,228]. A possible explanation for this phenomenon, linking Sdhd mutations and potassium channel function, could be an (increased) production of ROS by defective SDH in *Sdhd* +/- mice, however, this has not yet been clearly demonstrated[184].

In heterozygous *Sdhd* mutants, SDH activity is reduced, but no specific disease phenotype develops, although a slight carotid body hyperplasia has been observed[184,227,228]. Apparently, in *Sdhd* +/- mice the loss of one *Sdhd* allele is sufficiently compensated by transcription from the wild type allele in order to escape paraganglioma formation[227]. In addition, a *Sdhd/H19* double knockout mouse model also did not show increased paraganglioma or pheochromocytoma susceptibility, and H19 may thus, at least in mice, not be a modifier gene in paraganglioma development as proposed in the model for parent-of-origin-dependent inheritance (see paragraph 3.4: 'inheritance of head and neck paraganglioma syndromes')[227]. On the other hand, the lack of paraganglioma formation in Sdhd knockout mice may also be explained by a great number of other unknown physiological or genetic factors, and differences in genotype-phenotype correlations between mouse and man are not uncommon[227]. Furthermore, inducible tissue-specific *Sdhd* knockouts are not yet available and the effects of heterozygous germ-line *Sdhd* defects and subsequent LOH in paraganglion tissue have not been studied[227].

*Vhl*

In Vhl knockout mice, the loss of both *Vhl* alleles (*Vhl* -/-) results in embryonic lethality during mid-gestation, due to lack of placental vasculogenesis[229,230]. A specific homozygous *Vhl* mutation at codon 200 is compatible with life and results in mild polycythemia, which resembles the homozygous recessive *VHL* mutation causing hereditary polycythemia in man (Chuvash syndrome)[229]. Heterozygous *Vhl* knockout mice (*Vhl* +/-) develop cavernous liver hemangiomas and sometimes renal cysts (both rarely associated with *VHL* mutations in humans), but paragangliomas or pheochromocytomas are not reported[226,229,230]. The reason for this phenotypic divergence between mouse and man is currently unknown.

*Nf1*

Like *Sdhd* and *Vhl*, the *Nf1* null state (*Nf1* -/-) is lethal in mice[231]. Heterozygous knockout mice (*Nf1* +/-) do not develop neurofibromas and astrocytomas, the hallmark tumors of human *NF1* mutations, but heterozygous mutations involving exon 31 of the mouse *Nf1* gene have been associated with pheochromocytoma formation[226,231]. In the Nf1-linked tumors, the wild type *Nf1* allele is lost, indicating that *Nf1* is a pheochromocytoma tumor suppressor gene in mice[226]. Microarray gene expression studies of *Nf1* knockout pheochromocytomas show an expression signature of early central and peripheral nervous system development, in line with the concept of persistent neuronal precursor cells and disrupted developmental culling as a cause of pheochromocytoma formation (see paragraph 4.3: 'abnormal development of neuronal precursor cells')[232].

*Ret*

The human MEN type 2B syndrome, consisting of medullary thyroid carcinoma, pheochromocytoma, mucosal neuroma and marfanoid skeletal changes, is predominantly caused by one specific mutation at codon 918 in exon 16 of the human *RET* gene (see paragraph 3.1: 'NF1, RET and VHL')[95]. The corresponding Ret mutation in mice produces a phenotype with comparable lesions in the mouse thyroid and adrenal medulla[233]. Homozygous ($Ret^{MEN2B}/Ret^{MEN2B}$) mice develop bilateral adrenal chief cell hyperplasia early in life, which invariably progresses to pheochromocytoma[233]. A minority of heterozygous mutants ($Ret^{MEN2B}/+$) also develop adrenal chief cell hyperplasia (in 16%) and pheochromocytomas (in 2%), but later in life[233]. Interestingly, extra-adrenal chief cell nodules were also observed in homozygous mutant mice, but the head and neck region was not investigated in this model[233]. The $Ret^{MEN2B}/Ret^{MEN2B}$ mice display the highest frequency of pheochromocytoma formation of any mouse model to date. Based on observations in the $Ret^{MEN2B}$ mouse model, it has been hypothesized that gain-of-function Ret mutations such as $Ret^{MEN2B}$ cause adrenal tumors through abnormal migration, proliferation and survival of neuronal precursor cells (see paragraph 4.3: 'abnormal development of neuronal precursor cells')[233].

# 5. Historical notes

The major steps, and the people that took them in the development of our current understanding of the paraganglion system and the neoplasms that stem from it, deserve some special consideration, because (in the words of Goethe): "*die Geschichte einer Wissenschaft ist die Wissenschaft selbst*".

## 5.1 The discovery of the carotid body

The discovery of the carotid body and its recognition as an anatomical entity is widely attributed to Albrecht von Haller (1708-1777), who named it the 'exiguum caroticum', although it was his student Taube who in 1743 published the first anatomical description of what he called the 'ganglion minutem'[234,235].

*Albrecht von Haller (1708-1777)*

Von Haller was a Swiss scholar and veritable 'homo universalis', with contributions in the fields of anatomy, medicine, botany, physiology, philosophy, politics and poetry. He started his studies in medicine in 1724 in Tübingen, but went to Leiden in 1725 to continue under the famous Herman Boerhaave. While in Leiden, he also studied anatomy and surgery with Bernhard Siegfried Albinus. In 1727, at the age of eighteen, he graduated doctor medicinae under Boerhaave after writing a thesis on an otolaryngological topic, proving that a recently discovered 'salivary duct' was in fact a blood vessel. He wrote an extensive seven-volume book about his learning in Leiden: 'Erläuterungen zu Boerhaaves Institutiones'. He later went on to study in London, Oxford, Paris, and Basel before he was eventually appointed chair of medicine, anatomy, botany and surgery at the University of Göttingen in 1736. Von Haller was an avid researcher and a prolific writer. The body of

work he produced was immense, covering all fields of human knowledge, most notably botany, anatomy and poetry. His most famous contributions to the field of medicine were his recognition of the mechanism of respiration, the autonomous function of the heart, and the description of nerve and muscle activity. Another great passion, botany, led him to mountaineer in the Alps in search of specimen for his enormous plant collection and botanic garden. These forays inspired him to write his most famous poem, called 'Die Alpen', the first example of lyrical appreciation of the natural beauty of the high mountains in European literature[236,237]. At the time, the relation between high altitude and hyperplasia of the carotid body had yet to be discovered.

### 5.2 The discovery of the carotid body function

After the discovery of the carotid body and its description in 1743, it has taken considerable time before its function was elucidated. The first to hypothesize that the carotid body had chemoreceptive properties was De Castro, who in 1926 stated that the glomus caroticum could 'taste the blood'[238]. It was Corneille J.F. Heymans, a Flemish physiologist, who in the 1920's was the first to fully appreciate the function of the carotid body as a peripheral chemoreceptor of oxygen, carbon dioxide and acidity in the arterial blood and its role in the reflexogenic regulation of ventilation and blood pressure[5].

*Corneille J.F. Heymans (1892-1968)*

The discovery of the carotid body as a regulator of ventilation occurred rather by chance, as Heymans later claimed, by doing a 'foolish experiment' at the end of a day experimenting on the severed heads of dogs. Spurred on by his father and principle teacher to '*never kill an animal at the end of an experiment if the animal may still be used for any experimental purpose.. even if it looks foolish*', he injected some cyanide that happened to be standing

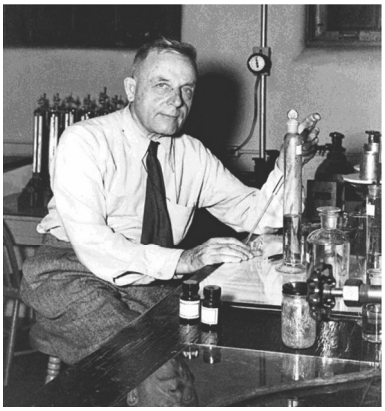
on the laboratory desk into the carotid artery of a dog with- and a dog without intact innervation of the carotid body area, and was surprised by the difference in ventilatory response. This observation lead Heymans to perform the experiments that ultimately won him the Nobel Prize in 1938[239,240].

### 5.3 The Warburg controversy

In 1926, the biochemist Otto Warburg observed that hypoxia alone was not sufficient to kill cancer cells, and that even in aerobic conditions when oxidative phosphorylation would be more efficient, they display high rates of glycolysis, a process he called 'aerobic glycolysis'[241]. For this discovery of 'the nature and mode of action of the respiratory enzyme', he was awarded the Nobel Prize in 1931. Warburg believed that the metabolic shift form oxidative phosphorylation to glycolysis in cancer cells, now called the 'Warburg effect', was the fundamental cause of cancer (in the words of Warburg at a lecture in of Nobel-Laureates in 1966: "*Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar*")[241,242]. The contention that mitochondrial dysfunction and disrupted metabolism is the 'only one prime cause of cancer' was disputed by many (as summarized by one of Warburg's most prominent opponents, the biochemist Sidney Weinhouse: "*at present the whole conception of cancer initiation or survival by "faulty" respiration and high glycolysis seems too simplistic for serious consideration*" and "*it has led far too many researchers into dead-end avenues of fruitless, ill-conceived attempts at the understanding or treatment of the neoplastic process*")[243]. With the discovery of genetic mechanisms and environmental factors as causes of cancer, Warburg's hypothesis on the origin of cancer faded to the background, much to the dismay of its discoverer, who in 1956 even stated: "*…there is today no other explanation for the origin of cancer cells, either special or general. From this point of view, mutation and carcinogenic agents are not alternatives, but empty words, unless metabolically specified. Even more harmful in the struggle against cancer can be the continual discovery of miscellaneous cancer agents and cancer viruses, which, by obscuring the underlying phenomena, may hinder necessary preventive measures and thereby become responsible for cancer cases*"[242]. Warburg died in 1970, at a time when his hypothesis was largely replaced by the idea that cancer was caused by abnormalities in genes and gene expression, not metabolism.

Although nowadays it is widely accepted that mitochondrial dysfunction is not the 'only one prime cause of cancer', as Warburg stated, it is also recognized that a high rate of glycolysis is a feature of many cancer types, a characteristic that today is exploited by the FDG-PET imaging of tumors (see paragraph 2.1: 'paragangliomas of the head and neck'). Indeed, in

the 2011 revision of their influential publication 'The hallmarks of cancer', Hanahan and Weinberg acknowledge the ability of cancer cells to reprogram energy metabolism as an 'emerging hallmark of cancer'[185,217]. What is more, the identification of defects in the mitochondrial SDH as a cause of paragangliomas and pheochromocytomas, as well as the identification in 2002 of defects in fumarate hydratase (FH), another TCA cycle component, as a cause of leiomyoma, leiomyosarcoma, and clear cell renal carcinoma, have confirmed Warburg's' hypothesis that defects in the cellular metabolism can actually initiate tumor growth. These discoveries have contributed to the revival of the scientific interest in the role of the metabolism in the neoplastic cell, both as a possible cause of cancer as well as a potential therapeutic target, just as Warburg envisioned[176,177,185,197,244].



*Otto Warburg (1883-1970)*

## 6. Outline of the thesis

The aim of this thesis is to gain insight in the genetics, inheritance and tumor biology of head and neck paragangliomas and the clinical consequences for paraganglioma patients, with a focus on hereditary paraganglioma syndrome in the Netherlands.

**Chapter one** consists of a general introduction into the current insights in head and neck paragangliomas, the diagnosis and treatment, the causative genes and their phenotypes, the heredity of paraganglioma syndromes, and an attempt is made to link gene mutations to tumor formation and behavior through the molecular biology of paragangliomas.

In **chapter two**, the current insights in the genetics of paragangliomas are reviewed, with an emphasis on the most recent developments.

In **chapter three**, the mutation frequency of SDH genes in the Netherlands is analyzed, using the data acquired by the Department of Human Genetics and the Laboratory for DNA Diagnostics of the LUMC, the primary Dutch national referral center for SDH mutation scanning. Using this SDH mutation database we evaluate the relative role of each of the SDH genes in the Dutch paraganglioma and pheochromocytoma population and the contribution of Dutch SDH founder mutations.

In **chapter four**, the clinical characteristics of Dutch head and neck paraganglioma patients treated at the Leiden University Medical Center (LUMC) are evaluated and correlated to their gene mutation status. It describes the unusual genetic make-up of the Dutch head and neck paraganglioma population and the consequences for the clinical characteristics of paraganglioma syndrome in the Netherlands.

In **chapter five**, the phenotype of the SDHD.D92Y (Asp92Tyr) Dutch founder mutation, the most prominent cause of paraganglioma syndrome in the Netherlands, is studied in a large multigenerational paraganglioma family, with a focus on the penetrance and the risk of developing symptomatic disease.

In **chapter six**, gene expression of SDHAF2- (formerly known as the PGL2 locus) and SDHD-linked paragangliomas as well as sporadic head and neck paragangliomas is investigated using RNA-microarrays, a high-throughput gene expression profiling technique. An attempt is made to distinguish these genetic subgroups on the basis of their gene expression profile and to link mutations in *SDHD* and *SDHAF2* to specific tumorigenic pathways.

In **chapter seven**, the unusual parent-of-origin-dependent inheritance that is observed in SDHD-linked paraganglioma kindreds is further investigated. A hypothesis is put forward that explains the exclusive paternal transmission of paragangliomas in SDHD-linked families, a pattern consistent with maternal imprinting, in the absence of imprinting of the *SDHD* gene itself.

**Chapter eight** consist of a summary of the thesis, its general implications for head and neck paragangliomas in the Netherlands and future perspectives of paraganglioma research.

## Abbreviations

| | |
|---|---|
| CT | computed tomography |
| FAD | flavin-adenine-dinucleotide, cofactor of SDHA |
| HO-2 | hemoxygenase 2 |
| HIF | hypoxia inducible factor |
| HRCT | high resolution computed tomography |
| KIF1B | kinesin family member 1B |
| LOH | loss of heterozygosity |
| MAPK | mitogen-activated protein kinase |
| MEN | multiple endocrine neoplasia |
| MRI | magnetic resonance imaging |
| mTOR | mammalian target of rapamycin |
| NF1 | neurofibromatosis type 1, may also refer to neurofibromin 1 gene or protein |
| NOS | nitric oxide synthetase |
| PET | positron emission tomography |
| PHD | prolyl hydroxylase |
| RET | proto-oncogene (REarranged during Transfection) |
| ROS | reactive oxygen species |
| SDH | succinate dehydrogenase; complex II in the electron transport chain |
| SDHA | succinate dehydrogenase subunit A; catalytic flavoprotein subunit of SDH |
| SDHAF2 | succinate dehydrogenase assembly factor 2; factor in the flavination of SDHA |
| SDHB | succinate dehydrogenase subunit B; catalytic iron sulphur subunit of SDH |
| SDHC | succinate dehydrogenase subunit C; anchoring subunit of SDH |
| SDHD | succinate dehydrogenase subunit D; anchoring subunit of SDH |
| TCA | tricarboxylic acid (cycle), or Krebs cycle |
| TMEM127 | transmembrane protein 127, may refer to gene or protein |
| VHL | Von Hippel-Lindau, may refer to the VHL syndrome, gene or protein |

# References


1. Tischler AS, Paraganglia. In: Histology for pathologists. Raven Press ltd., New York. (1992) 363-369.
2. Zak FG, Lawson W, The paraganglionic chemoreceptor system. Springer-Verlag Inc., New York. (1982).
3. Heath D, The human carotid body in health and disease. J Pathol. 164 (1991) 1-8.
4. Lack EE, Cubilla AL, Woodruff JM, Paragangliomas of the head and neck region. Pathology study of tumors from 71 patients. Hum Pathol. 10 (1979) 191-218.
5. Heymans C, Bouckaert JJ, Dautrebande L, Carotid sinus and respiratory reflexes. II. Reflex respiratory influences of the acidosis, of the carbonic anhydride, of the hydrogen ion and of the anoxemia. Carotid sinuses and respiratory exchanges in the lungs and seaward of the lungs. Arch Int Pharmacodyn Ther. 39 (1930) 400-448.
6. Tischler AS, Pheochromocytoma and extra-adrenal paraganglioma: updates. Arch Pathol Lab Med. 132 (2008) 1272-1284.
7. Neumann HPH, Eng C, The approach to the patient with paraganglioma. J Clin Endocrinol Metab. 94 (2009) 2677-2683.
8. Neumann HPH, Pheochromocytoma. In: Harrison's Principles of Internal Medicine, 17th edition. McGraw-Hill Companies Inc., New York. (2008) 2269-2275.
9. Kale SS, Rao VK, Bentz ML, Glomus tumor of the index finger. J Craniofac Surg. 17 (2006) 801-804.
10. Schipper J, Boedeker CC, Maier W, Neumann HPH, Paragangliomas in the head-/neck region I: Classification and diagnosis. HNO 52 (2004) 569-574.
11. Strong VE, Kennedy T, Al-Ahmadie H et al., Prognostic indicators of malignancy in adrenal pheochromocytomas: clinical, histopathologic, and cell cycle/apoptosis gene expression analysis. Surgery 143 (2008) 759-768.
12. Douwes Dekker PB, Corver WE, Hogendoom PCW et al., Multiparameter DNA flow-sorting demonstrates diploidy and SDHD wild-type gene retention in the sustentacular cell compartment of head and neck paragangliomas: chief cells are the only neoplastic component. J Pathol. 202 (2004) 456-462.
13. Batsakis J, Chemodectomas of the head and neck. In: Tumors of the head and neck. Clinical and pathological considerations, 2nd edition. Williams & Wilkins, Baltimore. (1979) 280-288.
14. Oosterwijk JC, Jansen JC, Van Schothorst EM et al., First experiences with genetic counselling based on predictive DNA diagnosis in hereditary glomus tumours (paragangliomas). J Med Genet. 33 (1996) 379-383.
15. Van der Kleij-Corssmit EPM, Havekes B, Vriends AHJT et al., Pheochromocytomas and paragangliomas: implications of new insights for diagnosis and treatment. Ned Tijdschr Geneeskd. 152 (2008) 489-493.
16. Baysal BE, Hereditary paraganglioma target's diverse paraganglia. J Med Genet. 39 (2002) 617-622.
17. Hermsen MA, Sevilla MA, Llorente JL et al., Relevance of germline mutation screening in both familial and sporadic head and neck paraganglioma for early diagnosis and clinical management. Cell Oncol. 32 (2010) 275-283.
18. Erickson D, Kudva YC, Ebersold MJ et al., Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab. 86 (2001) 5210-5216.
19. Hensen EF, Jansen JC, Siemers MD et al., The Dutch founder mutation SDHD.D92Y shows a reduced penetrance for the development of paragangliomas in a large multigenerational family. Eur J Hum Genet. 18 (2010) 62-66.
20. Van den Berg R, Imaging and management of head and neck paragangliomas. Eur Radiol. 15 (2005) 1310-1318.
21. Gjuric M, Gleeson M, Consensus statement and guidelines on the management of paragangliomas of the head and neck. Skull Base 19 (2009) 109-116.
22. Mendenhall WM, Amdur RJ, Vaysberg M et al, Head and neck paragangliomas. Head Neck 33 ((2011) 1530-1534.
23. Raisanen J, Shapiro B, Glazer GM et al., Plasma catecholamines in pheochromocytoma: effect of urographic contrast media. Am J Roentgenol. 143 (1984) 43-46.

24. Bessell-Browne R, O'Malley ME, CT of pheochromocytoma and paraganglioma: risk of adverse events with i.v. administration of nonionic contrast material. Am J Roentgenol. 188 (2007) 970-974.
25. Baid SK, Lai EW, Wesley RA et al., Brief communication: radiographic contrast infusion and catecholamine release in patients with pheochromocytoma. Ann Intern Med. 150 (2009) 27-32.
26. Van den Berg R., Schepers A, De Bruine FT et al., The value of MR angiography techniques in the detection of head and neck paragangliomas. Eur J Radiol. 52 (2004) 240-245.
27. Chen H, Sippel RS, O'Dorisio MS et al., The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas 39 (2010) 775-783.
28. Kantorovich V, King KS, Pacak K, SDH-related pheochromocytoma and paraganglioma. Best Pract Res Clin Endocrinol Metab 24 (2010) 415-424.
29. Petri BJ, Van Eijck CH, de Herder WW et al., Phaeochromocytomas and sympathetic paragangliomas. Br J Surg. 96 (2009) 1381-1392.
30. Havekes B, Lai EW, Corssmit EPM et al., Detection and treatment of pheochromocytomas and paragangliomas: current standing of MIBG scintigraphy and future role of PET imaging. Q.J.Nucl.Med.Mol. Imaging 52 (2008) 419-429.
31. Varma K, Jain S, Mandal S, Cytomorphologic spectrum in paraganglioma. Acta Cytol. 52 (2008) 549-556.
32. Fleming MV, Oertel YC, Rodriguez ER, Fidler WJ, Fine-needle aspiration of six carotid body paragangliomas. Diagn Cytopathol. 9 (1993) 510-515.
33. Shamblin WR, ReMine WH, Sheps SG, Harrison EG Jr., Carotid body tumor (chemodectoma). Clinicopathologic analysis of ninety cases. Am.J.Surg. 122 (1971) 732-739.
34. Makeieff M, Raingeard I, Alric P et al., Surgical management of carotid body tumors. Ann Surg Oncol. 15 (2008) 2180-2186.
35. Lim JY, Kim J, Kim SH et al., Surgical treatment of carotid body paragangliomas: outcomes and complications according to the shamblin classification. Clin Exp Otorhinolaryngol. 3 (2010) 91-95.
36. Fisch U, Infratemporal fossa approach for glomus tumors of the temporal bone. Ann Otol Rhinol Laryngol. 91 (1982) 474-479.
37. Moe KS, Li D, Linder TE et al., An update on the surgical treatment of temporal bone paraganglioma. Skull Base Surg. 9 (1999) 185-194.
38. Sanna M, Fois P, Pasanisi E, et al., Middle ear and mastoid glomus tumors (glomus tympanicum): an algorithm for the surgical management. Auris Nasus Larynx 37 (2010) 661-668.
39. Bradshaw JW, Jansen JC, Management of vagal paraganglioma: is operative resection really the best option? Surgery 137 (2005) 225-228.
40. Watkins LD, Mendoza N, Cheesman AD, Symon L, Glomus jugulare tumours: a review of 61 cases. Acta Neurochir (Wien) 130 (1994) 66-70.
41. Papaspyrou K, Mann WJ, Amedee RG, Management of head and neck paragangliomas: review of 120 patients. Head Neck 31 (2009) 381-387.
42. Makek M, Franklin DJ, Zhao JC, Fisch U, Neural infiltration of glomus temporale tumors. Am J Otol. 11 (1990) 1-5.
43. Teppema LJ, Dahan A, The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. Physiol Rev. 90 (2010) 675-754.
44. Weiss KL, Wax MK, Haydon RC et al., Intracranial pressure changes during bilateral radical neck dissections. Head Neck 15 (1993) 546-552.
45. McGovern PJ Jr., Swan KG, Management of bilateral internal jugular venous injuries. Injury 16 (1985) 259-260.
46. Zeitler DM, Glick J, Har-El G, Preoperative embolization in carotid body tumor surgery: is it required? Ann Otol Rhinol Laryngol. 119 (2010) 279-283.
47. Liapis C, Gougoulakis A, Karydakis V et al., Changing trends in management of carotid body tumors. Am Surg. 61 (1995) 989-993.
48. Li J, Wang S, Zee C, Yang J et al., Preoperative angiography and transarterial embolization in the management of carotid body tumor: a single-center, 10-year experience. Neurosurgery 67 (2010) 941-948.

49. Watanabe D, Tanabe A, Naruse M et al., Transcatheter arterial embolization for the treatment of liver metastases in a patient with malignant pheochromocytoma. Endocr J. 53 (2006) 59-66.
50. Krauel L, Albert A, Mora J et al., Use of angioembolization as an effective technique for the management of pediatric solid tumors. J Pediatr Surg. 44 (2009) 1848-1855.
51. Chino JP, Sampson JH, Tucci DL et al., Paraganglioma of the head and neck: long-term local control with radiotherapy. Am J Clin Oncol. 32 (2009) 304-307.
52. Foote RL, Pollock BE, Gorman DA et al., Glomus jugulare tumor: tumor control and complications after stereotactic radiosurgery. Head Neck 24 (2002) 332-338.
53. Huy PT, Kania R, Duet M et al., Evolving concepts in the management of jugular paraganglioma: a comparison of radiotherapy and surgery in 88 cases. Skull.Base. 19 (2009) 83-91.
54. Ramsden RT, Bulman CH, Lorigan BP, Osteoradionecrosis of the temporal bone. J Laryngol Otol. 89 (1975) 941-955.
55. Lustig LR, Jackler RK, Lanser MJ, Radiation-induced tumors of the temporal bone. Am J Otol. 18 (1997) 230-235.
56. Hawthorne MR, Makek MS, Harris JP, Fisch U, The histopathological and clinical features of irradiated and nonirradiated temporal paragangliomas. Laryngoscope 98 (1988) 325-331.
57. Chen PG, Nguyen JH, Payne SC et al., Treatment of glomus jugulare tumors with gamma knife radiosurgery. Laryngoscope 120 (2010) 1856-1862.
58. Jansen JC, Van den Berg R, Kuiper A et al., Estimation of growth rate in patients with head and neck paragangliomas influences the treatment proposal. Cancer 88 (2000) 2811-2816.
59. Van der Mey AGL, Frijns JHM, Cornelisse CJ et al., Does Intervention Improve the Natural Course of Glomus Tumors - A Series of 108 Patients Seen in A 32-Year Period. Ann Otol Rhinol Laryngol. 101 (1992) 635-642.
60. Pacak K, Linehan WM, Eisenhofer G et al., Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. Ann Intern Med. 134 (2001) 315-329.
61. Harding JL, Yeh MW, Robinson BG et al., Potential pitfalls in the diagnosis of phaeochromocytoma. Med.J.Aust. 182 (2005) 637-640.
62. Benn DE, Gimenez-Roqueplo AP, Reilly JR et al., Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab. 91 (2006) 827-836.
63. Walz MK, Alesina PF, Wenger FA et al., Laparoscopic and retroperitoneoscopic treatment of pheochromocytomas and retroperitoneal paragangliomas: results of 161 tumors in 126 patients. World J.Surg. 30 (2006) 899-908.
64. Brunt LM, Lairmore TC, Doherty GM et al., Adrenalectomy for familial pheochromocytoma in the laparoscopic era. Ann Surg. 235 (2002) 713-720.
65. Brunt LM, Minimal access adrenal surgery. Surg Endosc. 20 (2006) 351-361.
66. Walz MK, Peitgen K, Diesing D et al., Partial versus total adrenalectomy by the posterior retroperitoneoscopic approach: early and long-term results of 325 consecutive procedures in primary adrenal neoplasias. World J Surg. 28 (2004) 1323-1329.
67. Neumann HPH, Bender BU, Reincke M et al., Adrenal-sparing surgery for pheochromocytoma. Br J Surg. 86 (1999) 94-97.
68. Yip L, Lee JE, Shapiro SE et al., Surgical management of hereditary pheochromocytoma. J.Am.Coll.Surg. 198 (2004) 525-534.
69. Lairmore TC, Ball DW, Baylin SB, Wells SA,Jr., Management of pheochromocytomas in patients with multiple endocrine neoplasia type 2 syndromes. Ann Surg. 217 (1993) 595-601.
70. Walz MK, Petersenn S, Koch JA et al., Endoscopic treatment of large primary adrenal tumours. Br J Surg. 92 (2005) 719-723.
71. Fliedner SM, Lehnert H, Pacak K, Metastatic paraganglioma. Semin Oncol. 37 (2010) 627-637.
72. Adjalle R, Plouin PF, Pacak K, Lehnert H, Treatment of malignant pheochromocytoma. Horm Metab Res. 41 (2009) 687-696.
73. Zografos GN, Vasiliadis G, Farfaras AN et al., Laparoscopic surgery for malignant adrenal tumors. JSLS. 13 (2009) 196-202.
74. Colen TY, Mihm FG, Mason TP, Roberson JB, Catecholamine-secreting paragangliomas: recent progress in diagnosis and perioperative management. Skull Base 19 (2009) 377-385.

75. Van der Horst-Schrivers A.N., Osinga TE, Kema IP et al., Dopamine excess in patients with head and neck paragangliomas. Anticancer Res. 30 (2010) 5153-5158.
76. Eisenhofer G, Siegert G, Kotzerke J et al., Current progress and future challenges in the biochemical diagnosis and treatment of pheochromocytomas and paragangliomas. Horm Metab Res. 40 (2008) 329-337.
77. Unger N, Pitt C, Schmidt IL et al., Diagnostic value of various biochemical parameters for the diagnosis of pheochromocytoma in patients with adrenal mass. Eur J Endocrinol. 154 (2006) 409-417.
78. Lenders JW, Eisenhofer G, Mannelli M, Pacak K, Phaeochromocytoma. Lancet 366 (2005) 665-675.
79. Galetta F, Franzoni F, Bernini G et al., Cardiovascular complications in patients with pheochromocytoma: a mini-review. Biomed Pharmacother. 64 (2010) 505-509.
80. Lee JH, Barich F, Karnell LH et al., National Cancer Data Base report on malignant paragangliomas of the head and neck. Cancer 94 (2002) 730-737.
81. Ayala-Ramirez M, Feng L, Johnson MM et al., Clinical Risk Factors for Malignancy and Overall Survival in Patients with Pheochromocytomas and Sympathetic Paragangliomas: Primary Tumor Size and Primary Tumor Location as Prognostic Indicators. J Clin Endocrinol Metab. 96 (2011) 717-725.
82. Chapman DB, Lippert D, Geer CP et al., Clinical, histopathologic, and radiographic indicators of malignancy in head and neck paragangliomas. Otolaryngol Head Neck Surg. 143 (2010) 531-537.
83. Eisenhofer G, Goldstein DS, Sullivan P, Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab. 90 (2005) 2068-2075.
84. John H, Ziegler WH, Hauri D, Jaeger P, Pheochromocytomas: can malignant potential be predicted? Urology 53 (1999) 679-683.
85. Van Duinen N, Steenvoorden D, Kema IP et al., Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. J Clin Endocrinol Metab. 95 (2010) 209-214.
86. Moskovic DJ, Smolarz JR, Stanley D et al., Malignant head and neck paragangliomas: is there an optimal treatment strategy? Head Neck Oncol. 2 (2010) 23.
87. Plouin PF, Amar L, Lepoutre C, Phaeochromocytomas and functional paragangliomas: Clinical management. Best Pract Res Clin Endocrinol Metab. 24 (2010) 933-941.
88. Andersen KF, Altaf R, Krarup-Hansen A et al., Malignant pheochromocytomas and paragangliomas - the importance of a multidisciplinary approach. Cancer Treat Rev. 37 (2011) 111-119.
89. Papewalis C, Kouatchoua C, Ehlers M et al., Chromogranin A as potential target for immunotherapy of malignant pheochromocytoma. Mol Cell Endocrinol. 335 (2011) 69-77.
90. Grogan RH, Mitmaker EJ, Duh QY, Changing paradigms in the treatment of malignant pheochromocytoma. Cancer Control 18 (2011) 104-112.
91. Joshua AM, Ezzat S, Asa SL et al., Rationale and evidence for sunitinib in the treatment of malignant paraganglioma/pheochromocytoma. J Clin Endocrinol Metab. 94 (2009) 5-9.
92. Jimenez C, Cabanillas ME, Santarpia L et al., Use of the tyrosine kinase inhibitor sunitinib in a patient with von Hippel-Lindau disease: targeting angiogenic factors in pheochromocytoma and other von Hippel-Lindau disease-related tumors. J Clin Endocrinol Metab. 94 (2009) 386-391.
93. Kulke MH, Stuart K, Enzinger PC et al., Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol. 24 (2006) 401-406.
94. Amar L, Baudin E, Burnichon N et al., Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. J Clin Endocrinol Metab 92 (2007) 3822-3828.
95. Opocher G, Schiavi F, Genetics of pheochromocytomas and paragangliomas. Best Pract Res Clin Endocrinol Metab. 24 (2010) 943-956.
96. Bausch B, Borozdin W, Mautner VF et al., Germline NF1 mutational spectra and loss-of-heterozygosity analyses in patients with pheochromocytoma and neurofibromatosis type 1. J Clin Endocrinol Metab. 92 (2007) 2784-2792.
97. DeAngelis LM, Kelleher MB, Post KD, Fetell MR, Multiple paragangliomas in neurofibromatosis: a new neuroendocrine neoplasia. Neurology 37 (1987) 129-133.
98. Boedeker CC, Erlic Z, Richard S et al., Head and neck paragangliomas in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. J Clin Endocrinol Metab. 94 (2009) 1938-1944.

99. Wohllk N, Schweizer H, Erlic Z et al., Multiple endocrine neoplasia type 2. Best Pract Res Clin Endocrinol Metab. 24 (2010) 371-387.
100. Machens A, Brauckhoff M, Holzhausen HJ et al., Codon-specific development of pheochromocytoma in multiple endocrine neoplasia type 2. J Clin Endocrinol Metab. 90 (2005) 3999-4003.
101. Kaelin WG, Von Hippel-Lindau disease. Annu Rev Pathol. 2 (2007) 145-173.
102. Barontini M, Dahia PL, VHL disease. Best Pract Res Clin Endocrinol Metab. 24 (2010) 401-413.
103. Mannelli M, Castellano M, Schiavi F et al., Clinically guided genetic screening in a large cohort of italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab. 94 (2009) 1541-1547.
104. Baysal BE, Ferrell RE, Willett-Brozick JE et al., Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287 (2000) 848-851.
105. Van Schothorst EM, Jansen JC, Bardoel AF et al., Confinement of PGL, an imprinted gene causing hereditary paragangliomas, to a 2-cM interval on 11q22-q23 and exclusion of DRD2 and NCAM as candidate genes. Eur J Hum Genet. 4 (1996) 267-273.
106. Heutink P, Van der Mey AGL, Sandkuijl LA et al., A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet. 1 (1992) 7-10.
107. Heutink P, Van Schothorst EM, Van der Mey AGL et al., Further localization of the gene for hereditary paragangliomas and evidence for linkage in unrelated families. Eur J Hum Genet. 2 (1994) 148-158.
108. Ricketts CJ, Forman JR, Rattenberry E et al., Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat. 31 (2010) 41-51.
109. Neumann HPH, Pawlu C, Peczkowska M et al., Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 292 (2004) 943-951.
110. Burnichon N, Rohmer V, Amar L et al., The succinate dehydrogenase genetic testing in a l a r g e prospective series of patients with paragangliomas. J Clin Endocrinol Metab. 94 (2009) 2817-2827.
111. Hensen EF, Siemers MD, Jansen JC et al., Mutations in SDHD are the major determinants of the clinical characteristics of Dutch head and neck paraganglioma patients. Clin Endocrinol. 75 (2011) 650-655.
112. Havekes B, Corssmit EPM, Jansen JC et al., Malignant paragangliomas associated with mutations in the succinate dehydrogenase D gene. J Clin Endocrinol Metab. 92 (2007) 1245-1248.
113. Van Houtum WH, Corssmit EPM, Douwes Dekker PB et al., Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol. 152 (2005) 87-94.
114. Hensen EF, Jordanova ES, Van Minderhout IJHM et al., Somatic loss of maternal chromosome 11 causes parent-of-origin-dependent inheritance in SDHD-linked paraganglioma and phaeochromocytoma families. Oncogene 23 (2004) 4076-4083.
115. Van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ et al., Genomic Imprinting in Hereditary Glomus Tumors - Evidence for New Genetic Theory. Lancet 2 (1989) 1291-1294.
116. Struycken PM, Cremers CWRJ, Mariman ECM et al., Glomus tumours and genomic imprinting: Influence of inheritance along the paternal or maternal line. Clin Otolaryngol. 22 (1997) 71-76.
117. Pigny P, Vincent A, Cardot BC et al., Paraganglioma after maternal transmission of a succinate dehydrogenase gene mutation. J Clin Endocrinol Metab. 93 (2008) 1609-1615.
118. Taschner PEM, Jansen JC, Baysal BE et al., Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer 31 (2001) 274-281.
119. Dannenberg H, Dinjens WN, Abbou M et al., Frequent germ-line succinate dehydrogenase subunit D gene mutations in patients with apparently sporadic parasympathetic paraganglioma. Clin Cancer Res. 8 (2002) 2061-2066.
120. Niemann S, Muller U, Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nature Genetics 26 (2000) 268-270.
121. Schiavi F, Boedeker CC, Bausch B et al., Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. JAMA 294 (2005) 2057-2063.
122. Peczkowska M, Cascon A, Prejbisz A et al., Extra-adrenal and adrenal pheochromocytomas associated with a germline SDHC mutation. Nat Clin Pract Endocrinol Metab. 4 (2008) 111-115.

123. Mannelli M, Ercolino T, Giache V et al., Genetic screening for pheochromocytoma: should SDHC gene analysis be included? J Med Genet. 44 (2007) 586-587.
124. Pasini B, Stratakis CA, SDH mutations in tumorigenesis and inherited endocrine tumours: lesson from the phaeochromocytoma-paraganglioma syndromes. J.Intern.Med. 266 (2009) 19-42.
125. Hensen EF, Van Duinen N, Jansen JC et al., High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands. Clin. Genet. 81 (2012) 284-288.
126. Astuti D, Latif F, Dallol A et al., Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet. 69 (2001) 49-54.
127. Timmers HJ, Kozupa A, Eisenhofer G et al., Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. J Clin Endocrinol Metab. 92 (2007) 779-786.
128. Schiavi F, Milne RL, Anda E et al., Are we overestimating the penetrance of mutations in SDHB? Hum Mutat. 31 (2010) 761-762.
129. Bayley JP, Grimbergen AE, Van Bunderen PA et al., The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet. 10 (2009) 34.
130. Solis DC, Burnichon N, Timmers HJ et al., Penetrance and clinical consequences of a gross SDHB deletion in a large family. Clin Genet. 75 (2009) 354-363.
131. Hes FJ, Weiss MM, Woortman SA et al., Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. BMC Med Genet. 11 (2010) 92.
132. Hao HX, Khalimonchuk O, Schraders M et al., SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 325 (2009) 1139-1142.
133. Kunst HP, Rutten MH, De Monnink JP et al., SDHAF2 (PGL2-SDH5) and Hereditary Head and Neck Paraganglioma. Clin Cancer Res. 17 (2011) 247-254.
134. Bayley JP, Kunst HP, Cascon A et al., SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol. 11 (2010) 366-372.
135. Van Baars FM, Van den Broek P, Cremers CWRJ, Veldman JE, Familial Non-Chromaffinic Paragangliomas (Glomus Tumors) - Clinical Aspects. Laryngoscope 91 (1981) 988-996.
136. Baysal BE, Lawrence EC, Ferrell RE, Sequence variation in human succinate dehydrogenase genes: evidence for long-term balancing selection on SDHA. BMC Biology 5 (2007) 12.
137. Pagnamenta AT, Hargreaves IP, Duncan AJ et al., Phenotypic variability of mitochondrial disease caused by a nuclear mutation in complex II. Mol Genet Metab. 89 (2006) 214-221.
138. Burnichon N, Briere JJ, Libe R et al., SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet. 19 (2010) 3011-3020.
139. Horvath R, Abicht A, Holinski-Feder E et al., Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). J Neurol Neurosurg Psychiatry 77 (2006) 74-76.
140. Hensen EF, Bayley JP, Recent advances in the genetics of SDH-related paraganglioma and pheochromocytoma. Fam Cancer 10 (2011) 355-363.
141. Ardehali H, Chen Z, Ko Y, Mejia-Alvarez R, Marban E, Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K+ channel activity. Proc Natl Acad Sci USA. 101 (2004) 11880-11885.
142. Edstrom E, Mahlamaki E, Nord B et al., Comparative genomic hybridization reveals frequent losses of chromosomes 1p and 3q in pheochromocytomas and abdominal paragangliomas, suggesting a common genetic etiology. Am J Pathol. 156 (2000) 651-659.
143. Qin Y, Yao L, King EE et al., Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet. 42 (2010) 229-233.
144. Neumann HPH, Sullivan M, Winter A et al., Germline Mutations of the TMEM127 Gene in Patients with Paraganglioma of Head and Neck and Extraadrenal Abdominal Sites. J Clin Endocrinol Metab. 96 (2011) E1279-1282.
145. Yao L, Schiavi F, Cascon A et al., Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. JAMA 304 (2010) 2611-2619.

146. Ladroue C, Carcenac R, Leporrier M et al., PHD2 mutation and congenital erythrocytosis with paraganglioma. N Engl J Med. 359 (2008) 2685-2692.
147. Percy MJ, Zhao Q, Flores A et al., A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc Natl Acad Sci USA. 103 (2006) 654-659.
148. Yeh IT, Lenci RE, Qin Y et al., A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumors. Hum Genet. 124 (2008) 279-285.
149. Schlisio S, Kenchappa RS, Vredeveld LC et al., The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. Genes Dev. 22 (2008) 884-893.
150. Lee S, Nakamura E, Yang H et al., Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. Cancer Cell 8 (2005) 155-167.
151. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F et al., Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet. 43 (2011) 663-667.
152. Jimenez RH, Lee JS, Francesconi M et al., Regulation of gene expression in hepatic cells by the mammalian Target of Rapamycin (mTOR). PLoS One. 5 (2010) e9084.
153. Jafri M, Maher E, The genetics of phaeochromocytoma – using clinical features to guide genetic testing. Eur J Endocrinol. 166 (2011) 151-158.
154. Vaque JP, Fernandez-Garcia B, Garcia-Sanz P et al., c-Myc inhibits Ras-mediated differentiation of pheochromocytoma cells by blocking c-Jun up-regulation. Mol Cancer Res. 6 (2008) 325-339.
155. Badenhop RF, Jansen JC, Fagan PA et al., The prevalence of SDHB, SDHC, and SDHD mutations in patients with head and neck paraganglioma and association of mutations with clinical features. J Med Genet. 41 (2004) e99.
156. Baysal BE, Willett-Brozick JE, Lawrence EC et al., Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J Med Genet. 39 (2002) 178-183.
157. Baysal BE, Willett-Brozick JE, Lawrence EC et al., Genetic heterogeneity in hereditary paraganglioma (PGL): SDHD is the primary locus in imprinted PGL pedigrees. Am J Hum Genet. 67 (2000) 83.
158. Baysal BE, McKay S, Kim YJ et al., Genomic imprinting at a boundary element flanking the SDHD locus. Hum Mol Genet. 20 (2011) 4452-4461.
159. Dannenberg H, de Krijger RR, Zhao JM et al., Differential loss of chromosome 11q in familial and sporadic parasympathetic paragangliomas detected by comparative genomic hybridization. Am J Pathol. 158 (2001) 1937-1942.
160. Yamashita R, Usui T, Hashimoto S, et al., Predominant expression of mutated allele of the succunate dehydrogenase D (SDHD) gene in the SDHD-related paragangliomas. Endocr J. 56 (2009) 1129-1135.
161. Riemann K, Sotlar K, Kupka S et al., Chromosome 11 monosomy in conjunction with a mutated SDHD initiation codon in nonfamilial paraganglioma cases. Cancer Genet.Cytogenet. 150 (2004) 128-135.
162. Yoshimizu T, Miroglio A, Ripoche MA et al., The H19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci USA. 105 (2008) 12417-12422.
163. Neumann HPH, Erlic Z, Maternal transmission of symptomatic disease with SDHD mutation: fact or fiction? J Clin Endocrinol Metab. 93 (2008) 1573-1575.
164. Yeap PM, Tobias ES, Mavraki E et al., Molecular Analysis of Pheochromocytoma after Maternal Transmission of SDHD Mutation Elucidates Mechanism of Parent-of-Origin Effect. J Clin Endocrinol Metab. 96 (2011) E2009-2013.
165. Baldisserotto M, Peletti AB, DeAngelo M et al., Beckwith-Wiedemann syndrome and bilateral adrenal pheochromocytoma: sonography and MRI findings. Pediatr Radiol. 35 (2005) 1132-1134.
166. Bemurat L, Gosse P, Ballanger P et al., Successful laparoscopic operation of bilateral pheochromocytoma in a patient with Beckwith-Wiedemann syndrome. J Hum Hypertens. 16 (2002) 281-284.
167. Tycko B, Epigenetic gene silencing in cancer. J Clin Invest 105 (2000) 401-407.
168. Margetts CDE, Astuti D, Gentle DC et al., Epigenetic analysis of HIC1, CASP8, FLIP, TSP1, DCR1, DCR2, DR4, DR5, KvDMR1, H19 and preferential 11p15.5 maternal-allele loss in von Hippel-Lindau and sporadic phaeochromocytomas. Endoc Relat Cancer 12 (2005) 161-172.
169. Lui WO, Chen JD, Glasker S et al., Selective loss of chromosome 11 in pheochromocytomas associated with the VHL syndrome. Oncogene 21 (2002) 1117-1122.

170. Hering A, Guratowska M, Bucsky P et al., Characteristic genomic imbalances in pediatric pheochromocytoma. Genes Chromosomes.Cancer 45 (2006) 602-607.
171. Fournet JC, Mayaud C, De Lonlay P et al., Unbalanced expression of 11p15 imprinted genes in focal forms of congenital hyperinsulinism - Association with a reduction to hamozygosity of a mutation in ABCC8 or KCNJ11. Am J Pathol. 158 (2001) 2177-2184.
172. Douwes Dekker PB, Hogendoorn PCW, Kuipers-Dijkshoorn N et al, SDHD mutations in head and neck paragangliomas result in destabilization of complex II in the mitochondrial respiratory chain with loss of enzymatic activity and abnormal mitochondrial morphology. J Pathol. 201 (2003) 480-486.
173. Gimenez-Roqueplo AP, Favier J, Rustin P et al., The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. Am J Hum Genet. 69 (2001) 1186-1197.
174. Pasini B, McWhinney SR, Bei T et al., Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. Eur J Hum Genet. 16 (2008) 79-88.
175. Gottlieb E, Tomlinson IP, Mitochondrial tumour suppressors: a genetic and biochemical update. Nat Rev Cancer 5 (2005) 857-866.
176. King A, Selak MA, Gottlieb E, Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 25 (2006) 4675-4682.
177. Kaelin WG, Jr., SDH5 mutations and familial paraganglioma: somewhere Warburg is smiling. Cancer Cell 16 (2009) 180-182.
178. Albayrak T, Scherhammer V, Schoenfeld N et al., The tumor suppressor cybL, a component of the respiratory chain, mediates apoptosis induction. Mol Biol Cell 14 (2003) 3082-3096.
179. Ishii T, Yasuda K, Akatsuka A et al., A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. Cancer Res. 65 (2005) 203-209.
180. Yankovskaya V, Horsefield R, Tornroth S et al., Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299 (2003) 700-704.
181. Baysal BE, Clinical and molecular progress in hereditary paraganglioma. J Med Genet. 45 (2008) 689-694.
182. Valko M, Izakovic M, Mazur M et al., Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell Biochem. 266 (2004) 37-56.
183. Semenza GL, Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 29 (2010) 625-634.
184. Piruat JI, Pintado CO, Ortega-Saenz P et al., The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. Mol Cell Biol. 24 (2004) 10933-10940.
185. Hanahan D, Weinberg RA, Hallmarks of cancer: the next generation. Cell 144 (2011) 646-674.
186. Kim JW, Dang CV, Multifaceted roles of glycolytic enzymes. Trends Biochem.Sci. 30 (2005) 142-150.
187. Bayley JP, Devilee P, Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? Curr Opin Genet Dev. 20 (2010) 324-329.
188. Baysal BE, On the association of succinate dehydrogenase mutations with hereditary paraganglioma. Trends Endocrinol Metab. 14 (2003) 453-459.
189. Lemarie A, Huc L, Pazarentzos E et al., Specific disintegration of complex II succinate:ubiquinone oxidoreductase links pH changes to oxidative stress for apoptosis induction. Cell Death.Differ. 18 (2011) 338-349.
190. Saitoh Y, Ouchida R, Miwa N, Bcl-2 prevents hypoxia/reoxygenation-induced cell death through suppressed generation of reactive oxygen species and upregulation of Bcl-2 proteins. J Cell Biochem. 90 (2003) 914-924.
191. Shimizu S, Eguchi Y, Kosaka H et al., Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-xL. Nature 374 (1995) 811-813.
192. Kunz M, Ibrahim SM, Molecular responses to hypoxia in tumor cells. Mol Cancer 2 (2003) 23.
193. Douwes Dekker PB, Kuipers-Dijkshoorn N, Hogendoorn PCW et al. G2M arrest, blocked apoptosis, and low growth fraction may explain indolent behavior of head and neck paragangliomas. Hum Pathol. 34 (2003) 690-698.

194. Wang DG, Johnston CF, Barros D'Sa AA, Buchanan KD, Expression of apoptosis-suppressing gene BCL-2 in human carotid body tumours. J Pathol. 183 (1997) 218-221.
195. McClintock DS, Santore MT, Lee VY et al., Bcl-2 family members and functional electron transport chain regulate oxygen deprivation-induced cell death. Mol Cell Biol. 22 (2002) 94-104.
196. Pollard PJ, El Bahrawy M, Poulsom R et al., Expression of HIF-1 alpha, HIF-2 alpha (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. J Clin Endocrinol Metabol. 91 (2006) 4593-4598.
197. Dong LF, Jameson VJ, Tilly D et al., Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. J.Biol.Chem. 286 (2011) 3717-3728.
198. Arias-Stella J, Valcarcel J, The human carotid body at high altitudes. Pathol Microbiol (Basel) 39 (1973) 292-297.
199. Arias-Stella J, Valcarcel J, Chief cell hyperplasia in the human carotid body at high altitudes; physiologic and pathologic significance. Hum Pathol. 7 (1976) 361-373.
200. Lopez-Barneo J, Ortega-Saenz P, Pardal R et al., Carotid body oxygen sensing. Eur Respir J. 32 (2008) 1386-1398.
201. Prabhakar NR, Oxygen sensing by the carotid body chemoreceptors. J Appl Physiol. 88 (2000) 2287-2295.
202. Lahiri S, Roy A, Baby SM et al., Oxygen sensing in the body. Prog Biophys Mol Biol. 91 (2006) 249-286.
203. Wilson DF, Roy A, Lahiri S, Immediate and long-term responses of the carotid body to high altitude. High Alt Med Biol. 6 (2005) 97-111.
204. Semenza GL, HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev. 20 (2010) 51-56.
205. Liu LP, Simon MC, Regulation of transcription and translation by hypoxia. Cancer Biol Ther. 3 (2004) 492-497.
206. Hirota K, Semenza GL, Regulation of angiogenesis by hypoxia-inducible factor 1. Crit Rev Oncol Hematol. 59 (2006) 15-26.
207. Choi KS, Bae MK, Jeong JW et al., Hypoxia-induced angiogenesis during carcinogenesis. J Biochem Mol Biol. 36 (2003) 120-127.
208. Mansfield KD, Guzy RD, Pan Y et al., Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. Cell Metab. 1 (2005) 393-399.
209. Pollard PJ, Briere JJ, Alam NA et al., Accumulation of Krebs cycle intermediates and over-expression of HIF1 alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet. 14 (2005) 2231-2239.
210. Selak MA, Armour SM, MacKenzie ED et al., Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 7 (2005) 77-85.
211. Mahon PC, Hirota K, Semenza GL, FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 15 (2001) 2675-2686.
212. Favier J, Gimenez-Roqueplo AP, Pheochromocytomas: the (pseudo)-hypoxia hypothesis. Best Pract Res Clin Endocrinol Metab. 24 (2010) 957-968.
213. Dahia PLM, Ross KN, Wright ME et al., A HIF1 alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. Plos Genet. 1 (2005) 72-80.
214. Van Nederveen FH, Dannenberg H, Sleddens HF et al., p53 alterations and their relationship to SDHD mutations in parasympathetic paragangliomas. Mod Pathol. 16 (2003) 849-856.
215. Bunz F, Dutriaux A, Lengauer C et al., Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282 (1998) 1497-1501.
216. Brieger J, Bedavanija A, Gosepath J et al., Vascular endothelial growth factor expression, vascularization and proliferation in paragangliomas. ORL J Otorhinolaryngol Relat Spec. 67 (2005) 119-124.
217. Hanahan D, Weinberg RA, The hallmarks of cancer. Cell 100 (2000) 57-70.
218. Acharya A, Das I, Chandhok D, Saha T, Redox regulation in cancer: a double-edged sword with therapeutic potential. Oxid Med Cell Longev. 3 (2010) 23-34.
219. Douwes Dekker PB, Kuipers-Dijkshoorn NJ, Baelde HJ, et al., Basic fibroblast growth factor and fibroblastic growth factor receptor-1 may contribute to head and neck paraganglioma development by an autocrine or paracrine mechanism. Hum Pathol 38 (2007) 79-85.

220. Jyung RW, LeClair EE, Bernat RA et al., and R.S.Tuan, Expression of angiogenic growth factors in paragangliomas. Laryngoscope 110 (2000) 161-167.
221. Johannessen CM, Johnson BW, Williams SM et al., TORC1 is essential for NF1-associated malignancies. Curr.Biol. 18 (2008) 56-62.
222. Zhu J, Blenis J, Yuan J, Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. Proc Natl Acad Sci USA. 105 (2008) 6584-6589.
223. Dhillon AS, Hagan S, Rath O, Kolch W, MAP kinase signalling pathways in cancer. Oncogene 26 (2007) 3279-3290.
224. Guertin DA, Sabatini DM, An expanding role for mTOR in cancer. Trends Mol Med. 11 (2005) 353-361.
225. Wouters BG, Koritzinsky M, Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 8 (2008) 851-864.
226. Tischler AS, Powers JF, Alroy J, Animal models of pheochromocytoma. Histol Histopathol. 19 (2004) 883-895.
227. Bayley JP, Van Minderhout IJHM, Hogendoorn PCW et al., Sdhd and SDHD/H19 knockout mice do not develop paraganglioma or pheochromocytoma. PLoS.One. 4 (2009) e7987.
228. Ortega-Saenz P, Pascual A, Piruat JI, Lopez-Barneo J, Mechanisms of acute oxygen sensing by the carotid body: lessons from genetically modified animals. Respir Physiol Neurobiol. 157 (2007) 140-147.
229. Kapitsinou PP, Haase VH, The VHL tumor suppressor and HIF: insights from genetic studies in mice. Cell Death Differ. 15 (2008) 650-659.
230. Haase VH, The VHL tumor suppressor in development and disease: functional studies in mice by conditional gene targeting. Semin Cell Dev Biol. 16 (2005) 564-574.
231. Dasgupta B, Gutmann DH, Neurofibromatosis 1: closing the GAP between mice and men. Curr Opin Genet Dev. 13 (2003) 20-27.
232. Powers JF, Evinger MJ, Zhi J et al., Pheochromocytomas in Nf1 knockout mice express a neural progenitor gene expression profile. Neuroscience 147 (2007) 928-937.
233. Smith-Hicks CL, Sizer KC, Powers JF et al., C-cell hyperplasia, pheochromocytoma and sympathoadrenal malformation in a mouse model of multiple endocrine neoplasia type 2B. EMBO J. 19 (2000) 612-622.
234. Pick JC, The discovery of the carotid body. J Hist Med Allied Sci. 14 (1959) 61-73.
235. Taube HWL, De vera nervi intercostalis origine. Diss Inaug Göttingen (1743).
236. Bay JC, Albrecht Von Haller Medical Encyclopedist. Bull Med Libr Assoc. 48 (1960) 393-403.
237. Hintzsche E, (Victor) Albrecht von Haller. In: Complete Dictionary of Scientific Biography. www.encyclopedia.com. (2008).
238. De Castro F, Sur la structure et l'innervation de la glande intercarotiddienne (glomus caroticum) de l'homme et des mammiferes, et sur un nouveau systeme d'innervation autonome du nerf glossopharyngien. Etudes anatomiques et esperimentales. Trabajos del Laboratorio de investigaciones biologicas de la Universidad de Madrid 24 (1926) 365-432..
239. Heymans C, A Look at An Old But Still Current Problem. Ann Rev Physiol. 25 (1963) 1-14.
240. De Castro F, The discovery of sensory nature of the carotid bodies-invited article. Adv Exp Med Biol. 648 (2009) 1-18.
241. Warburg O, Wind F, Negelein E, The metabolism of tumors in the body. J Gen Physiol. 8 (1927) 519-530.
242. Warburg O, On the origin of cancer cells. Science 123 (1956) 309-314.
243. Weinhouse S, The Warburg hypothesis fifty years later. Z Krebsforsch Klin Onkol Cancer Res Clin Oncol. 87 (1976) 115-126.
244. Favier J, Briere JJ, Burnichon N et al., The Warburg effect is genetically determined in inherited pheochromocytomas. PLoS.One. 4 (2009) e7094.