Published in final edited form as:

Endocr Regul. 2011 April ; 45(2): 65–90.

PHEOCHROMOCYTOMA: A CATECHOLAMINE AND OXIDATIVE STRESS DISORDER

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Abstract

The WHO classification of endocrine tumors defines pheochromocytoma as a tumor arising from chromaffin cells in the adrenal medulla — an intra-adrenal paraganglioma. Closely related tumors of extra-adrenal sympathetic and parasympathetic paraganglia are classified as extra-adrenal paragangliomas. Almost all pheochromocytomas and paragangliomas produce catecholamines. The concentrations of catecholamines in pheochromocytoma tissues are enormous, potentially creating a volcano that can erupt at any time. Significant eruptions result in catecholamine storms called “attacks” or “spells”. Acute catecholamine crisis can strike unexpectedly, leaving traumatic memories of acute medical disaster that champions any intensive care unit. A very well-defined genotype-biochemical phenotype relationship exists, guiding proper and cost-effective genetic testing of patients with these tumors. Currently, the production of norepinephrine and epinephrine is optimally assessed by the measurement of their O-methylated metabolites, normetanephrine or metanephrine, respectively. Dopamine is a minor component, but some paragangliomas produce only this catecholamine or this together with norepinephrine. Methoxytyramine, the O-methylated metabolite of dopamine, is the best biochemical marker of these tumors. In those patients with equivocal biochemical results, a modified clonidine suppression test coupled with the measurement of plasma normetanephrine has recently been introduced. In addition to differences in catecholamine enzyme expression, the presence of either constitutive or regulated secretory pathways contributes further to the very unique mutation-dependent catecholamine production and release, resulting in various clinical presentations.

Oxidative stress results from a significant imbalance between levels of prooxidants, generated during oxidative phosphorylation, and antioxidants. The gradual accumulation of prooxidants due to metabolic oxidative stress results in proto-oncogene activation, tumor suppressor gene inactivation, DNA damage, and genomic instability. Since the mitochondria serves as the main source of prooxidants, any mitochondrial impairment leads to severe oxidative stress, a major outcome of which is tumor development. In terms of cancer pathogenesis, pheochromocytomas and paragangliomas represent tumors where the oxidative phosphorylation defect due to the mutation of succinate dehydrogenase is the cause, not a consequence, of tumor development. Any succinate dehydrogenase pathogenic mutation results in the shift from oxidative phosphorylation to aerobic glycolysis in the cytoplasm (also called anaerobic glycolysis if hypoxia is the main cause of such a shift). This phenomenon, also called the Warburg effect, is well demonstrated by a positive [¹⁸F]-fluorodeoxyglucose positron emission tomography scan. Microarray studies, genome-wide association studies, proteomics and protein arrays, metabolomics, transcriptomics, and bioinformatics approaches will remain powerful tools to further uncover the pathogenesis of

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DISCLOSURE STATEMENT: The author has nothing to disclose.

these tumors and their unique markers, with the ultimate goal to introduce new therapeutic options for those with metastatic or malignant pheochromocytoma and paraganglioma. Soon oxidative stress will be tightly linked to a multistep cancer process in which the mutation of various genes (perhaps in a logistic way) ultimately results in uncontrolled growth, proliferation, and metastatic potential of practically any cell. Targeting the mTORC, IGF-1, HIF and other pathways, topoisomerases, protein degradation by proteasomes, balancing the activity of protein kinases and phosphatases or even synchronizing the cell cycle before any exposure to any kind of therapy will soon become a reality. Facing such a reality today will favor our chances to “beat” this disease tomorrow.

Keywords

Pheochromocytoma; Paraganglioma; Catecholamines; Metanephrines; Mitochondria; Oxidative Phosphorylation; Glycolysis

Introduction

It is a great honor and pleasure to give such a highly recognized introductory Plenary Lecture at the 10th International Symposium on Catecholamines and Other Neurotransmitters in Stress. These symposia have been very successful over the past 30 years thanks to the extraordinary work and dedication of Richard Kvetnansky and his staff, as well as the participation of top scientists and clinicians in the field of catecholamines and stress. Every three years, surrounded by the exceptional Slovak's hospitality and the Smolenice castle history, these scientists and clinicians meet to discuss the newest advances and discoveries in stress. The history of this event is deeply, and in an unrepeatable way, linked to those who have made the best discoveries and greatest impact on catecholamine and stress research, notably Julie Axelrod who won the Nobel Prize in Physiology or Medicine in 1970. I dedicate this lecture to Richard Kvetnansky - without him the field of stress would be comparable to a child without parents, to my mentors, and to all patients whose suffering from stress- and catecholamine-related disorders has become much less pronounced due to the work of all of you. It does not matter the place we are destined to, as long as we have devotion and passion for what we do. Passion moves us to realization. We cannot do things without passion; without it there can only be mediocrity. It is my personal view that the 30-year extraordinary success of this International Symposium is a true testament to our passion and destiny.

Alfred Kohn, professor of histology at the Charles University in Prague, introduced the terms “chromaffin”, “chromaffin system”, “paraganglion”, and “paraganglionic cell” (1-5). The term “pheochromocytoma” was proposed by Pick in 1912 (6) and comes from the Greek words *phaios*, which means dusky (brown), and *chroma*, which means color, and refers to the staining that occurs when the tumors are treated with chromium salts. The brown pigment of the chromaffin reaction is composed of oxidation products of *epinephrine* (EPI) or *norepinephrine* (NE) resulting in the generation of adrenochrome and noradrenochrome, respectively. The first diagnosis of pheochromocytoma was made in 1886 by Fränkel (7) who found bilateral tumors of the adrenal gland on autopsy of a patient who had died suddenly after collapse.

The 2004 WHO classification of endocrine tumors defines pheochromocytoma as a tumor arising from chromaffin cells in the adrenal medulla — an intra-adrenal paraganglioma. Closely related tumors of extra-adrenal sympathetic and parasympathetic paraganglia are classified as extra-adrenal paragangliomas. Paragangliomas are divided into two groups: those that arise from parasympathetic-associated tissues (most commonly along cranial

nerves; e.g. glomus or carotid body tumors) and those that arise from sympathetic-associated chromaffin tissue (often designated extra-adrenal pheochromocytomas). For simplification, in this review, the term pheochromocytoma will be used to refer to both adrenal and sympathetic ganglia-derived extra-adrenal tumors.

In 1936, EPI was isolated from a pheochromocytoma by Kelly et al. (8). In 1949, Holton (9) first demonstrated the presence of NE in a pheochromocytoma. Early in the 1950's, von Euler showed that patients with pheochromocytoma had increased urine excretion of EPI, NE, or hydroxytyramine (metabolite of dopamine (DA)) (10). Shortly thereafter, Lund together with Moller described elevated plasma concentrations of NE and EPI in patients with pheochromocytoma (11, 12). Armstrong and co-workers were first in showing elevated urine excretion of vanillylmandelic acid (VMA) in patients with pheochromocytoma (13). In 1957 Axelrod and co-workers described O-methylation as the important pathways in catecholamine metabolism (14) and LaBrosse and co-workers for the first time demonstrated elevated urine excretion of *normetanephrine* (O-methylated metabolite of NE) in patients with pheochromocytoma (15).

According to different reviews and statistics, pheochromocytomas account for approximately 0.05% to 0.6% of patients with any degree of sustained hypertension (16-20). This accounts for only 40-50% of persons harboring the tumor, since about the same number of patients have only paroxysmal hypertension and up to 10% are normotensive. The current prevalence of sustained hypertension in the adult population of Western countries is up to 30% (21-23) and therefore, in Western countries the prevalence of this tumor can be estimated to lie between 1:4,500 to 1:1,700, with an annual incidence of 3-8 cases per 1 million per year in the general population (24).

1. Pheochromocytoma: A catecholamine disorder

1.1. Overview of catecholamine synthesis and metabolism

Understanding the clinical presentation of this tumor and its correct biochemical diagnosis, the reader should have the basic understanding of catecholamine synthesis, release, and metabolism. The sources of catecholamines and the pathways of their metabolism, an appreciation of how catecholamines are metabolized and released among different cells and tissues, including sympathetic neurons and chromaffin cells from which pheochromocytomas are derived, represent the most important information. Furthermore, confounding influences of medications that affect the disposition of catecholamines are other variables that often require important consideration in ruling out or confirming the presence of this tumor.

The rate-limiting step in catecholamine biosynthesis involves conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (**Figure 1**) (25). Sources of catecholamines are therefore principally dependent on the presence of this enzyme, which is largely confined to dopaminergic and noradrenergic neurons of the central nervous system, and to sympathetic nerves and adrenal and extra-adrenal chromaffin cells from which pheochromocytoma is derived. Other sites of catecholamine synthesis include certain non-neuronal cells of the gastrointestinal tract and kidneys (26, 27).

Conversion of DOPA to DA is catalyzed by aromatic L-amino acid decarboxylase, an enzyme with a wide tissue distribution. The DA formed in the cytoplasm by aromatic L-amino acid decarboxylase is transported into vesicular storage granules. In dopaminergic neurons, DA is released without any further conversion to NE; but in noradrenergic neurons and adrenal chromaffin cells DA is further converted to NE by dopamine β -hydroxylase. The enzyme is present in vesicular storage granules, either bound to the vesicular membrane

or present in the soluble matrix core. The noradrenergic neurochemical phenotype of pheochromocytoma depends on both translocation of DA into storage granules and the presence of dopamine β -hydroxylase.

The additional presence of phenylethanolamine N-methyltransferase (PNMT) in adrenal medullary chromaffin and pheochromocytoma cells leads to further conversion of NE to EPI. Since PNMT is a cytosolic enzyme, this step depends on leakage of NE from vesicular storage granules into the cell cytoplasm. After EPI is synthesized it is translocated back into chromaffin granules (28).

Catechol-O-methyltransferase (COMT) is responsible for a major pathway of catecholamine metabolism, catalyzing O-methylation of DA to methoxytyramine, NE to normetanephrine, and EPI to metanephrine, as well as methylation of 3,4-dihydroxyphenylglycol (DHPG) to 3-methoxy-4-hydroxyphenylglycol (MHPG) (29) (**Figure 2**). Under baseline conditions, normetanephrine and metanephrine are produced in small amounts and only at extraneuronal locations, with the single largest source representing adrenal chromaffin cells, which accounts for over 90% of circulating metanephrine and 24% to 40% of circulating normetanephrine (30). About 90% of the VMA formed in the body is produced in the liver, mainly from hepatic uptake and metabolism of circulating DHPG and MHPG (31). In humans, VMA and the sulfate and glucuronide conjugates of MHPG represent the main end products of NE and EPI metabolism and are eliminated mainly by urinary excretion.

1.2. Catecholamines and adrenoceptors

It is important to understand the function and organ-specific role of adrenoceptors to explain different organ responses to catecholamines often released in huge amounts from pheochromocytoma. Initially, adrenoceptors were divided into α and β types based on the rank order of potency of the various catecholamines in different vascular beds (32). Subsequent classification into α_1 , α_2 , β_1 , β_2 , β_3 , and dopamine D_{1-5} receptors has been based on ligand-binding studies and responses to synthetic agonists and antagonists (33-38).

α_1 -Adrenoceptors are postsynaptic and located on effector tissues such as vascular smooth muscle. Stimulation causes vasoconstriction and an increase in blood pressure, while stimulation of other α_1 -adrenoceptors can cause pupillary dilation, intestinal relaxation, and uterine contraction (**Table 1**) (39). The α_1 -antagonists are the most commonly targeted in therapy for treatment of hypertension due to catecholamine excess from pheochromocytoma (40).

Some α_2 -adrenoceptors are located presynaptically and inhibit NE secretion upon stimulation, while others on vascular smooth muscle are postsynaptic and extrasynaptic and cause vasoconstriction upon stimulation (**Table 1**) (39, 41, 42). Classic α_2 -agonists include clonidine, methyl dopa, and guanabenz. Central α_2 -agonist action in the brain suppresses sympathetic outflow and thereby reduces blood pressure, which is the basis for their pharmacologic use, commonly in patients with so-called pseudopheochromocytoma. The clonidine suppression test is the most important test to prove or rule out the presence of pheochromocytoma in patients with non-significantly elevated plasma NE or normetanephrine levels (43).

β_1 -Adrenoceptors have more diverse functions and stimulation of those in the heart causes positive inotropic and chronotropic actions, while stimulation of β_1 -adrenoceptors elsewhere causes lipolysis in fat cells, and increased renin secretion in the kidney (**Table 1**) (39). The β_1 -antagonists are the most commonly used drug in patients with tachyarrhythmia secondary to catecholamine excess in pheochromocytoma patients.

The stimulation of β_2 -adrenoceptors cause bronchodilation; vasodilation in some blood vessels, especially those in skeletal muscle; glycogenolysis (interestingly some patients with pheochromocytoma present with hyperglycemia of “unknown origin”); uterine and intestinal smooth muscle relaxation; and an increased release of NE from sympathetic nerves. Typical β_2 -agonists include metaproterenol, albuterol, terbutaline, and isoetharine. Propranolol, alprenolol, nadolol, and timolol are antagonists at both β_1 - and β_2 -adrenoceptors and are rarely used in patients with pheochromocytoma due to their non-selectivity that results in a more profound effect on brain function (e.g. tiredness, sleepiness).

While DA has very weak agonist activity at α - and β -adrenoceptors (clinically usually not appreciated), there are also specific D_{1-5} receptors to be aware of.

D_1 receptors are found mainly in coronary, renal, mesenteric, and cerebral vascular beds. Stimulation of these receptors causes vasodilation (44, 45). Stimulation of D_1 receptors in the kidney produces diuresis and natriuresis. As DA doses are increased, both β_1 - and α -adrenoceptors are stimulated, causing vasoconstriction and an increase in blood pressure. D_2 receptors are presynaptic on sympathetic nerve endings and stimulation inhibits release of NE. Other D_2 receptors are present on sympathetic ganglia and their stimulation inhibits ganglionic transmission, while others in the brain cause emesis and inhibit prolactin release (46). D_{3-5} receptors play a role in various brain functions (38).

In terms of specific catecholamine action on adrenoceptors, EPI has much more potent effects on β_2 -adrenoceptors than NE. Compared to NE, EPI also demonstrates greater or equal affinity for α_1 - and α_2 -adrenoceptors, and equivalent potency for β_1 -adrenoceptors. Due to the above differences, EPI exerts its effects on different populations of adrenoceptors than NE. As a circulating hormone, EPI acts potently on β_2 adrenoceptors of the skeletal muscle vasculature causing vasodilation. Metabolic effects of EPI include hyperglycemia, hyperlipidemia, thermogenesis, increased oxygen consumption, and hypokalemia. The hyperglycemic response to EPI is due to a series of actions, which include stimulation of hepatic glycogen phosphorylase, inhibition of glycogen synthase, stimulation of gluconeogenesis, inhibition of insulin secretion, and stimulation of glucagon release (47, 48). In contrast, NE released locally within the vasculature causes α_1 -adrenoceptor-mediated vasoconstriction. This and the chronotropic and inotropic effects of neurally released NE mediated by way of cardiac β_1 -adrenoceptors reflect a primary and critical function of the sympathoneural system in cardiovascular regulation, particularly maintenance of blood pressure. The above described differences in NE and EPI action and adrenoceptors translate into the clinical scenario. NE-secreting pheochromocytomas are mainly associated with hypertension, often with hypertensive crises if NE is released abruptly, while EPI-secreting tumors are more commonly associated with tachyarrhythmias.

Almost all tissues and organs in the body, some of them in a specific pattern, express adrenoceptors. Catecholamines also tend to decrease intestinal motility and tone and to inhibit intestinal secretion. Graded adrenergic activity stimulates renin secretion in the kidney and relaxation in the gallbladder, as well as relaxation of the detrusor muscle of the urinary bladder. There are also α_2 -adrenoceptor-mediated increases in platelet aggregation, and shunting of blood toward the cardiopulmonary region with stimulation of low-pressure cardiac baroreceptors (49, 50). Sweating of the palms of the hands and certain other sites, commonly referred to as adrenergic sweating, is due to α -adrenergic stimulation of apocrine glands.

Catecholamines diffusely inhibit gut motility and in some patients this can lead to hypodynamic ileus or “pseudo-obstruction.” Most endocrine organs are affected by catecholamines. Thus, stimulation of α -adrenoceptors tends to decrease the secretion of

most preformed hormones, whereas stimulation of β -adrenoceptors tends to stimulate release of preformed hormones. This includes insulin by the acinar cells of the pancreas, melatonin from the pineal, and antidiuretic hormone from the posterior pituitary.

Pheochromocytoma may also present with hypotension, commonly seen in patients harboring tumors that are secreting epinephrine or compounds causing vasodilatation, or after higher doses of antihypertensive therapy. Hypotension, postural or alternating, may occur secondary to hypovolemia, abnormal autonomic reflexes, or downregulation or differential stimulation of α - and β -adrenoceptors, or the type of co-secreted neuropeptide (e.g. neuropeptide Y) (18, 51, 52).

It should be noted that at least in about 20-30% of patients with very high and persistent levels of catecholamines adrenoceptor desensitization may occur (53). Such adrenoceptor downregulation may partially explain why some patients with pheochromocytoma are only moderately hypertensive despite high plasma levels of catecholamines (most often seen in patients with extensive metastatic disease).

1.3. Catecholamines and pheochromocytoma

Although practically all pheochromocytomas produce catecholamines, they present with significant variation in catecholamine content, depending on expression of biosynthetic enzymes (54-58), as well as the presence of either constitutive or regulated secretory pathways (59). Most pheochromocytomas produce predominantly NE (mainly those in extra-adrenal location), many produce both NE and EPI (typically adrenal tumors), and some produce predominantly EPI (virtually only adrenal tumors in patients with multiple endocrine neoplasia type 2 (MEN2) or neurofibromatosis type 1 (NF1)). Currently, the production of NE and EPI is optimally assessed by the measurement of their O-methylated metabolites, normetanephrine or metanephrine, respectively (**Figure 2**). DA, which is usually efficiently converted to NE, is a minor component, but some pheochromocytomas produce only this catecholamine or together with NE (e.g. some patients with succinate dehydrogenase (SDH) subunit B mutation (SDHB)) (60). Similar to normetanephrine and metanephrine, the O-methylated metabolite of DA, methoxytyramine, currently provides the most useful biochemical indication of the presence of tumors producing DA (61).

Activities of tyrosine hydroxylase, aromatic L-amino acid decarboxylase and dopamine β -hydroxylase are generally very high in these tumors, providing the basis for catecholamine overproduction in these tumors (62-64). Among different pheochromocytomas, activities of tyrosine hydroxylase are high in tumors from patients with MEN2 and low in those from von Hippel-Lindau (VHL) patients or patients with non-functional tumors (57, 58). In contrast, in a few tumors, mainly those related to SDHB, tyrosine hydroxylase may be absent (60).

In addition to differences in catecholamine enzyme expression, the presence of various secretory pathways contributes further to very unique mutation-dependent catecholamine production and release, resulting in various clinical presentations. For example, in patients with VHL syndrome, baseline catecholamine secretion is about 20-fold higher than in patients with MEN2 syndrome. However, using microarrays and proteomics data focusing on secretory pathway components, VHL tumors are characterized by the presence of a constitutive secretory pathway in contrast to MEN2 tumors, presenting with a regulated secretory pathway (**Figure 3**) (59). Translating these findings into clinical practice, patients with MEN2 pheochromocytoma present more often with paroxysmal hypertension, tachyarrhythmia, sweating, or other catecholamine-related symptoms or signs than patients with VHL tumors.

New observations confirmed definitively the existence of mutation-dependent biochemical phenotypes in various pheochromocytomas. Recently, Eisenhofer and co-workers carried out one of the largest studies to assess the value of metanephrines and methoxytyramine in the biochemical detection and of hereditary pheochromocytomas (65). One hundred seventy three patients with pheochromocytoma, including 38 with MEN2, 10 with NF1, 66 with VHL syndrome and 59 with mutations of genes for SDH type B or D were included. In contrast to patients with *VHL* and *SDH* mutations, all patients with MEN2 and NF1 presented with tumors characterized by increased plasma concentrations of metanephrine. VHL patients usually showed solitary increases in normetanephrine, whereas additional or solitary increases in methoxytyramine characterized 70% of patients with *SDH* mutations. Patients with NF1 and MEN2 could be discriminated from those with *VHL* and *SDH* mutations in 99% of cases by the combination of normetanephrine and metanephrine (**Table 2**). Measurement of plasma methoxytyramine discriminated patients with *SDH* mutations from those with *VHL* mutations in a further 78% of cases. As described previously, measurements of plasma and urinary catecholamines were less effective than plasma metanephrines for the correct classification of EPI- and NE-secreting tumors. It was concluded that distinct patterns of plasma metanephrines and methoxytyramine in patients with hereditary pheochromocytoma provide a very reliable approach to guide cost-effective genotyping of patients with these tumors. This data serves as an important platform for performance of appropriate genetic testing, especially in those patients presenting with apparently sporadic pheochromocytoma. Thus, from now, all genetic algorithms should incorporate results of biochemical testing into a cost-effective genetic testing of patients with pheochromocytoma.

The measurement of other plasma or urine metabolites further established specific hereditary pheochromocytoma catecholamine metabolomic phenotypes, especially two dominant clusters: (a) MEN2 and NF1 and (b) VHL and SDHx (65). Those clusters indicate the existence of distinct tumorigenesis pathways including mitochondrial dysfunction as described below.

NE is usually the predominant catecholamine produced by metastatic pheochromocytoma (66, 67), but these tumors are also often characterized by high tissue, plasma and urinary levels of DOPA and DA (68-72). However, elevations in plasma or urinary DOPA and DA are not sensitive or specific markers of benign or metastatic pheochromocytoma since they can also be found in patients with pheochromocytoma related to *SDHB* mutations. The recent introduction of methoxytyramine in the biochemical diagnosis of pheochromocytoma, including metastatic disease, is an excellent step forward in the advancement of earlier and more specific diagnose of these tumors (73). In contrast to much smaller increases in plasma DA levels, it has been found that plasma methoxytyramine levels are 200-370% higher in patients with metastatic pheochromocytoma than in those with non-metastatic tumors.

Expression of PNMT is controlled by glucocorticoid receptor-mediated mechanisms acting in concert with several transcription factors (74, 75). Local availability of steroids may explain why adrenal pheochromocytomas often produce EPI, whereas extra-adrenal tumors typically lack PNMT and produce predominantly NE (56). These observations may correspond well with an interesting clinical situation where, in patients with pheochromocytoma, acute administration of glucocorticoids (especially given intravenously) may cause significant catecholamine release, potentially resulting in a patient's death (76).

1.4. Pheochromocytoma and catecholamine excess and storm: Clinical implications

There are probably few names that haven't been used to describe pheochromocytoma – from somewhat complimenting “great masquerader” to unflattering “treacherous murderer”. These apparently “mixed feelings” relate to the rarity of pheochromocytoma in a population

of usual suspects – patients with poorly controlled and labile hypertension on one side and horrific devastation of acute catecholamine (“pheochromocytoma”) crisis on the other. Acute catecholamine crisis can strike unexpectedly resulting in serious emergency situations (**Table 3**) (17, 77, 78).

The concentrations of catecholamines in pheochromocytoma tissues are enormous (79), potentially creating a volcano that can erupt at any time. Significant eruptions result in catecholamine storms called “attacks” or “spells”. These can occur on a daily basis or infrequently, once per few weeks or months. Some of these “attacks” or “spells” are associated with specific triggers (e.g. stressful situation, exercise, drug-related (the worst one being Reglan used for nausea)) but in most of them, a trigger is not present. Smaller but continuous eruptions of catecholamines are also possible and almost always result in various symptoms and signs that are characteristic of adrenoceptor overstimulation by catecholamines (**Table 1**) (39). For example, in our series we found that in patients with sporadic NE-secreting pheochromocytomas, the average NE content was about 1,760,000 pg/g tissue with about 53% of its release each day. In sporadic EPI-secreting pheochromocytomas the average EPI content was about 3,801,000 pg/g tissue, with about 5% of its release each day (Eisenhofer, unpublished observations). At such levels, any direct tumor stimulation may lead to abrupt and significant catecholamine release that exceed normal plasma values 1,000 times or more with devastating consequences, often presenting as an emergency (**Table 3**) (80-82). These differences in catecholamine content and release as well as their action on adrenoceptors explain different clinical presentations and ultimately necessitate specific treatment for each patient.

However, in about 15-20% of patients with pheochromocytoma, basal plasma or urine catecholamines are within normal limits (83, 84). Some of these cases may be explained by the presence of so-called “*non-functional*” or “*silent*” *pheochromocytomas* that do not secrete significant amounts of catecholamines. Nevertheless, most of these “silent” tumors synthesize and metabolize catecholamines to metanephrines (elevated either in plasma or urine) and show elevations in plasma catecholamines only during paroxysmal attacks possessing the same danger as other pheochromocytomas (85, 86). In addition to catecholamines, pheochromocytomas are known to produce other vasoactive substances (neuropeptide Y, adrenomedullin, atrial natriuretic peptide) that may cause hypertension (83).

1.5. Current recommendations of biochemical diagnosis of pheochromocytoma: Special considerations on catecholamine metabolites

Missing a pheochromocytoma can have deadly consequences due to the detrimental effects of excess catecholamines, as previously outlined. Therefore, one of the most important considerations in the choice of an initial biochemical test is a high level of reliability that the test will provide a positive result in that rare patient with the tumor. Such a test also provides confidence that a negative result reliably excludes the tumor, thereby avoiding the need for multiple or repeat biochemical tests or even costly and unnecessary imaging studies to rule out the tumor. Therefore, the initial work-up of a patient with suspected pheochromocytoma should include a suitably sensitive biochemical test.

Previously and frequently performed measurements of urinary or plasma catecholamines do not provide reliable biochemical tests for detection of the tumor. This is particularly problematic due to intermittent catecholamine secretion in some patients, the presence of small tumors, or those tumors that release only DA.

Because metanephrines are continuously produced and continuously leak from pheochromocytoma tumor cells, largely independent of catecholamine release,

metanephrines have become the most optimal biochemical test in the diagnosis of this tumor (85). To minimize false-positive results, it is now our recommendation that biochemical testing samples always be collected in the quiescent state. For blood sampling we recommend that patients should be resting comfortably supine for at least 15-20 minutes before sampling.

In a landmark study, involving over 200 patients with pheochromocytoma and more than 600 patients in whom the tumor was excluded, measurements of plasma free metanephrines and urinary fractionated metanephrines provided the most highly sensitive diagnostic tests, urinary and plasma catecholamines offered intermediate diagnostic sensitivity, while urinary total metanephrines and VMA offered the least sensitivity (87). Analysis of receiver-operating characteristic curves showed that at equivalent levels of sensitivity the specificity of plasma free metanephrines was higher than that of all other tests, and that at equivalent levels of specificity the sensitivity of plasma free metanephrines was also higher than that of all other tests, even when the latter were combined (**Figure 4**). Consistent with these concepts it has now been established by several independent groups of investigators that measurements of plasma metanephrines provides superior diagnostic sensitivity over measurements of plasma or urinary catecholamines for detection of pheochromocytoma (87-90).

Several studies involving measurements of urinary fractionated metanephrines have similarly indicated that these tests provide superior diagnostic sensitivity over urinary or plasma catecholamines, urinary VMA, or total metanephrines, the latter measured as the combined sum of normetanephrine and metanephrine by early spectrophotometric methods (87, 91-94).

Due to the above considerations, a now widely endorsed recommendation is that initial biochemical testing should include measurements of plasma free or urinary fractionated metanephrines (95, 96). Both tests offer similarly high diagnostic sensitivity so that a negative result for either test appears equally effective for excluding pheochromocytoma. However, because of differences in specificity, tests of plasma free metanephrines may exclude pheochromocytoma in more patients without the tumor than do tests of urinary fractionated metanephrines. Nevertheless, any differences in overall diagnostic accuracy of plasma compared to urinary fractionated metanephrines are relatively small compared to differences of either test of metanephrines with tests of plasma or urinary catecholamines.

Measurements of plasma or urinary metanephrines may fail to detect some tumors that synthesize only small amounts of catecholamines or exclusively DA. Measurements of DA and its O-methylated metabolite, methoxytyramine, provide better methods for detecting DA-producing tumors than measurements of DA alone (61).

Due to the large numbers of patients tested for pheochromocytoma and the rarity of the tumor, false-positive results can be expected to outnumber true-positive results, even for tests with reasonably high specificity. The extent of increase of a positive test result is important to judging the likelihood of a pheochromocytoma. Increases in plasma concentrations of metanephrines 4x above the upper reference limit are extremely rare in patients without pheochromocytoma, but occur in about 80% of patients with the tumor (87). Providing biochemical test results are accurate, the likelihood of pheochromocytoma in such a patient is so high that the immediate task is to locate the tumor. However, up to about 30% of patients may have plasma metanephrine levels between the upper reference limit and 4x times above this limit. In these patients, before any localization of a tumor is attempted, additional testing or evaluation of a patient must be performed. First, any drug-induced effect on catecholamine and metanephrine levels must be taken into account; the reader

should be aware of the fact that practically all antihypertensive drugs (except calcium channel blockers) will elevate catecholamines and their metabolites (**Figure 5**) (40). But in many patients antihypertensive medication cannot be stopped and therefore, an additional test, called the clonidine suppression test, is usually carried out. The clonidine suppression test is safe, but unreliable in patients with normal or only mildly increased plasma catecholamine levels (97-99). Additional measurements of plasma normetanephrine before and after clonidine provide a method to overcome the above limitation in patients with elevated plasma concentrations of normetanephrine, but normal or mildly elevated plasma concentrations of NE (43). A decrease of plasma levels of normetanephrine of more than 40% or below the upper reference limit is present in almost all patients without pheochromocytoma, indicating a diagnostic specificity of 100%. This is similar to that for NE (specificity = 98%), where pheochromocytoma could be excluded by a decrease of NE of more than 50% or a level of NE after clonidine below the upper reference limit. Diagnostic sensitivity for normetanephrine responses to clonidine is about 98%, a substantial improvement over the sensitivity of only 67% for NE responses. Glucagon provocative test has a low sensitivity and is no longer recommended (100).

Simultaneous measurement of catecholamines and metanephrines can provide a very good platform to distinguish between sympathoadrenal and sympathoneuronal activation from the presence of pheochromocytoma. This is based on the findings that substantial amounts of metanephrine (>90%) and a much lower amount of normetanephrine (24%-40%) are normally produced within adrenal medullary cells independently of catecholamine release. Increases in metanephrines during sympathoadrenal or sympathoneuronal activation are smaller than increases in catecholamines. Thus, patients with false-positive results due to sympathoadrenal activation usually have larger percent increases of plasma NE than of plasma normetanephrine or of plasma EPI than of metanephrine.

2. Pheochromocytoma: An oxidative stress disorder

Oxidative phosphorylation is described as a metabolic cascade that uses energy released by the oxidation of various nutrients to produce adenosine triphosphate (ATP). Oxidative phosphorylation occurs in the mitochondria, where mitochondrial complexes transfer electrons from their donors to their acceptors (e.g. O_2). This process is often called the electron transport chain and energy released through this chain is used to transport protons (H^+). The proton transport occurs over the inner mitochondrial membrane to generate ATP. Oxidative phosphorylation is powered by the citric acid cycle (Krebs cycle) by the supply of NADH and succinate. Together, oxidative phosphorylation, the Krebs cycle, and the oxygen supply constitute the pivotal orchestra conducting the generation of ATP-dependent cell energy; without it the body would collapse and fail.

Oxidative phosphorylation is a highly efficient energy pathway, generating about 32-36 ATPs from every 1 molecule of glucose. Failure of any member of this orchestra leads to a decrease in the energy production. The most common failure is hypoxia, commonly seen in cancer patients. This is because in rapidly growing tumor cells the oxygen supply, despite significant angiogenesis (the generation of new vessels), does not match the very high demand of tumor cells for oxygen and nutrients. The second most common cause of failure is mitochondrial dysfunction, also commonly seen in many tumors, if not all of them (**Figure 6**) (101, 102). This results in the shift from oxidative phosphorylation to aerobic glycolysis in the cytoplasm (also called anaerobic glycolysis if hypoxia is the main cause of such a shift). Glycolysis results in inefficient ATP production (only 2 ATPs produced for every glucose molecule) and pyruvate and lactate production. This phenomenon is also called the Warburg effect, coined in the 1930s when Otto Warburg observed a significant increase in glycolysis and lactate production in tumors (102-104). Since he assumed that the

oxygen was not altered in the tumors he proposed that this phenomenon reflected mitochondrial dysfunction rather than true hypoxia. Although the rate of glycolysis can increase by 30x or even more, cells are put into a metabolic hardship followed by severe metabolic stress.

Currently, it is accepted that metabolic oxidative stress results from a significant imbalance between levels of prooxidants that are generated during oxidative phosphorylation (including e.g. superoxide and hydrogen peroxide) and antioxidants (105). The gradual accumulation of prooxidants due to metabolic oxidative stress results in proto-oncogene activation, tumor suppressor gene inactivation, DNA damage, genomic instability, and limitless replication (no arrest in G₁ phase) (106) (107). Since the mitochondria serves as the main source of prooxidants, any mitochondrial impairment leads to severe oxidative stress, a major outcome of which is tumor development (**Figure 6**).

2.1. Mitochondrial complexes

There are 5 mitochondrial complexes: NADH-coenzyme Q oxidoreductase (complex I), SDH (also known as succinate:ubiquinone oxidoreductase; complex II), cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV), and ATP synthase (complex V). Complexes I, III, and IV are proton pumping enzymes; complex II does not have such activity. Rather, complex II serves to funnel electrons derived from the Krebs cycle and oxidative phosphorylation (complex I) to complex III, giving this enzyme the role of the conductor of the energy production orchestra (108). In the Krebs cycle this enzyme catalyses the conversion of succinate to fumarate. SDH consists of 4 subunits as described below.

Subunit A (SDHA; flavoprotein) constitutes the catalytic core that oxidizes succinate to fumarate following the release of electrons that are then bound to its covalently-bound FAD moiety; subunit B (SDHB) is an iron-sulfur protein that further funnels electrons from subunit A to coenzyme Q (ubiquinone) via subunits C (SDHC) and D (SDHD). A heme group of the SDHC subunit accepts electrons and transfers them to SDHD subunit. Both SDHC and SDHD subunits have the ubiquinone binding site and transfer electron to it to generate ubiquinol. SDHC and SDHD subunits also anchor the entire complex to the inner mitochondrial membrane. Abnormal SDH function due to mutations of nuclear DNA encoding for one of its subunits results in two completely different phenotypes. Defects in SDHA cause a metabolic neurodegenerative disorder called Leigh syndrome, whereas *SDHB*, *SDHC* and *SDHD* mutations predispose to familial pheochromocytoma via different mechanisms (**Figure 7**). However, recently, the role of SDHA in the pathogenesis of pheochromocytoma has been observed and confirmed (109). This is further well supported by recent findings that the *SDHAF2* (formerly known as *SDH5*) encodes SDH complex assembly factor 2 (SDHAF2) to control flavination of SDHA in order to preserve proper function of the entire SDH complex (110). Thus, when the complex II is disrupted at SDHA, the succinate dehydrogenase activity is impaired, if entirely, a severe neurodegenerative changes in various organs occur. If the complex II is disrupted at other subunits, electron transfer is impaired (it is unknown whether various mutations may cause a different electron transfer via SDHB/C/D subunits) including the abnormal removal of electrons from the flavin group of SDHA. This may lead to superoxide generation through autooxidation of the reduced (electron rich) flavin group by O₂ (111). Superoxide inhibits prolyl hydroxylases resulting in the stabilization of hypoxia inducible factor 1 α (HIF-1 α).

Consistent with Knudson's well-accepted two-hit hypothesis in tumorigenesis involving a tumor suppressor gene, a heterozygous germline mutation in an *SDHx* gene is usually associated with somatic loss of the non-mutant (wild type) allele in a tumor, i.e. loss of heterozygosity (112). Any pathogenic mutation of SDH subunits results in the abolition of

SDH enzymatic activity and activation of the angiogenic, hypoxic, and apoptotic pathways by various mechanisms, including increased transcription of vascular endothelial growth factor, glycolytic enzymes, and histone demethylation (**Figure 7**) (113) (114).

Disruptions in SDH activity also results in the accumulation of succinate, causing chronic oxidative stress, as first reported by Ishii et al (115). Furthermore, elevated succinate levels promote the stabilization of the transcription factor HIF-1 α via inhibition of the prolyl hydroxylase Egl-nine (**Figure 7**) (116). HIF regulates the transcription of about 100 genes, many of them involved in tumorigenesis, especially those sensing “hypoxia” (117) and glycolysis (9 out of 10 enzymes in the glycolytic pathway) (118).

A common feature of VHL and SDH dysfunction is their ability to increase stabilization of HIF-1 α and HIF-2 α , evident from recent data, under normoxia (111, 119-121). It is well known that HIF- α together with HIF- β (expressed constitutively) bind to hypoxia-responsive element to regulate the expression of various genes. As described above these genes are most relevant to tumorigenesis (**Figure 7**).

In patients with the VHL gene mutation, VHL protein cannot be degraded (there is incorrect binding of VHL protein-elongin-cullin complex) and HIF-1 α accumulates. This similar process occurs under hypoxic conditions. Overaccumulation of HIF-1 α results in the overexpression of various genes (e.g. vascular endothelial growth factor (VEGF) that increases angiogenesis; glucose transporters such as GLUT 1 and 4, and perhaps others, that help deliver glucose to tumor cells; glycolytic enzymes that power glycolysis; platelet-derived growth factor (PDGF) that promote growth of tumor cells and surrounding structures). It also inhibits the conversion of pyruvate to acetyl-CoA (underpowers Krebs cycle) and oxidative phosphorylation via c-Myc repressor (122). In contrast, HIF-2 α promotes c-MYC activity, angiogenesis tumor proliferation and their aggressive behavior. Various results have been shown related to VHL and SDH and either HIF-1 α or HIF-2 α stabilization and tumorigenesis (111, 119, 123). Similar to the study of Guzy et al. (123) and the author's view the degree of reactive oxygen species accumulation as well as HIF-1 α stabilization are most likely pivotal in the initial development of these tumors and angiogenesis with HIF-2 α playing an important role in tumor progression (proliferation, angiogenesis, invasiveness) and perhaps even poor outcome if metastatic (**Figure 7**).

2.2. Pheochromocytoma and succinate dehydrogenase: same complex but different tumors

It is the author's personal view that there were three important discoveries, to a certain degree overlooked, which served as the pivotal basis for the discovery of the link between oxidative stress and pheochromocytoma. It was Aria-Stella who first observed that high altitude was associated with the presence of carotid body tumors (124, 125) and similar discoveries were made in animals (126, 127). Second, electron microscopic and enzymatic studies presented by Cornog et al. demonstrated plentiful giant mitochondria and elevated lactate dehydrogenase activity in a patient with extra-adrenal pheochromocytoma (128). More than 30 years later, Cerecer-Gil et al. demonstrated good evidence linking high altitude to the development of SDHB-related head and neck paraganglioma (129) and Housley et al. also demonstrated (130) the presence of giant mitochondria in renal cell carcinoma related to SDHB gene mutation. Third, Watanabe et al. demonstrated significantly decreased SDH activity in patients with adrenal pheochromocytoma and linked this observation to the presence of giant mitochondria in these patients (131). It was in 1992 when Heutink et al. first mapped the underlying gene at 11q for a hereditary head and neck paraganglioma (132). Then in 2000 Baysal et al., by analyzing families with head and neck paragangliomas, discovered the first SDH complex gene, SDHD (11q23), causing these tumors (133). Following these discoveries, it was then postulated that HIF plays an

important role linking the mitochondrial dysfunction to the pheochromocytoma development (134).

SDHB—Most SDHB-related pheochromocytomas arise in extra-adrenal locations and have a strong tendency for metastatic spread (135-138) (Table 1). Among 32 patients with SDHB-associated pheochromocytomas in a German/Polish cohort (139), 28% had an adrenal pheochromocytoma, 59% had an extra-adrenal abdominal or thoracic paraganglioma and 31% had a head or neck paraganglioma. Twenty-eight percent had multifocal tumors. In our own cohort of SDHB pheochromocytoma patients, as many as 96% presented with an extra-adrenal tumors (138).

The mean age at diagnosis of paraganglioma in *SDHB* mutation carriers in previous series was approximately 30 years (138, 139). However, initial tumors may occur between age five and 65 (138, 140, 141). In the International SDH Consortium population, age-related penetrance (the proportion of gene carriers manifesting the signs or symptoms of the disease by a given age) among SDHB mutation carriers is estimated to be around 45% by age 40 (136). However, our own observations suggest that such a high penetrance is less likely and it is estimated to be around 10-20% at most (Pacak et al; unpublished data).

Reported malignancy rates in patients with SDHB-associated paragangliomas vary from 34 to 97% versus 10% for pheochromocytoma in general (135, 136, 138, 142, 143). In patients in whom a primary tumor originates in abdominal location with subsequent development of metastatic disease, there is a 40-50% chance that the disease is related to a SDHB gene mutation (137). Currently, it is unknown why SDHB-related pheochromocytoma has such a high rate of malignancy but since the SDHB subunit serves as the “gatekeeper” funneling electrons to complex III, this may serve as one of many explanations.

The diagnosis of SDHB-related pheochromocytoma might sometimes be delayed due to atypical clinical presentations since these tumors can either secrete only DA or can be biochemically silent (60). Thus, a large number of patients present with pain and other problems related to the space-occupying effects and invasive growth of the tumor, rather than to signs and symptoms related to catecholamine excess. Furthermore, there are excellent studies showing that *SDHB* mutations are associated with the presence of clear cell carcinoma and perhaps papillary thyroid carcinoma (144-147). It should be noted that kidney cancer may develop a long time before pheochromocytoma occurs and vice versa.

At present time all studies have failed to show any genotype-phenotype correlations (148). In addition, clinical phenotypes may differ largely between family members with the same *SDHB* mutation. However, recent studies show that patients with metastatic disease who developed their primary tumor before age 20 have disease most commonly related to a *SDHB* mutation (Pacak et al., unpublished data).

SDHD—SDHD mutations are mainly associated with head and neck paragangliomas, most often multiple, or multiple extra-adrenal pheochromocytomas (149, 150). Among 34 patients with SDHD-associated paragangliomas in a German/Polish cohort (139), 74% had multifocal paragangliomas, 39% had an extra-adrenal abdominal or thoracic paragangliomas and 79% had head or neck paragangliomas. The predominant location in the head and neck was confirmed by another other consortium (89%) (136).

Head and neck paragangliomas were for a long time presented as biochemically silent tumors but recent studies show that these tumors, at least in about 20-30% patients, produce catecholamines, mainly DA (151). Rare cases of SDHD-associated metastatic head and neck paragangliomas have been described (152). Additionally, in contrast to other

pheochromocytomas, these tumors often do not express cell membrane norepinephrine transporter system and therefore are not good candidates for [^{131}I]-MIBG treatment (153). Our preliminary data suggest, that [^{18}F]-fluorodopa positron emission tomography is the optimal imaging methods for these tumors (King et al., unpublished observations). As in SDHB-related pheochromocytomas, a genotype-phenotype correlation does not exist. Patients with SDHD-related pheochromocytomas present in earlier age at the first diagnosis compared to those with sporadic tumors. In contrast to SDHB gene mutation carriers where clear cell carcinoma of kidney is found in up to about 14% of patients, in SDHD carriers this number does not exceed 6% (146). Finally, inheritance of the SDHD gene shows maternal imprinting.

SDHC—SDHC mutations are mainly associated with the development of head and neck paragangliomas. To our knowledge, only 15 SDHC mutation carriers have been identified worldwide (154-157). Within a population-based international registry of patients with head and neck paragangliomas, the prevalence of SDHC mutations was 4%, versus 7% and 17% for SDHB, and SDHD, respectively (158). Among large groups of patients with adrenal or extra-adrenal pheochromocytomas, only very rarely were SDHC mutations identified (135, 155). The clinical behavior of SDHC-related paraganglioma appears similar to that of benign sporadic tumors and malignancy is extremely rare.

SDHA and other susceptibility genes—In the recent studies it has been demonstrated that SDHA, SDHAF2 (encodes co-factor to support SDHA function), and isocitrate dehydrogenase (catalyzes the oxidative decarboxylation of isocitrate to α -ketoglutarate, which is the Krebs cycle metabolite that serves as an essential co-substrate for prolylhydroxylase activity) are rare causes of pheochromocytoma (109, 110, 159). Testing for these genes is suggested if the testing for major susceptibility genes turns to be negative.

2.3. Pheochromocytoma and ATP production: The final step for energy release

During the past two decades more evidence for an important role of ATP synthase dysregulation in cancer has been presented. In various cancers, up- or down-regulation of specific ATP synthase subunits has been documented (160-162). Additionally, its localization on the cell surface of tumor cells and endothelial cells (163, 164) has been observed.

ATP synthase is a ubiquitous enzyme complex found in the mitochondrial inner membrane. It utilizes the proton gradient across the inner mitochondrial membrane, built by the complexes of the electron transfer chain, to catalyze the final step in the mitochondrial oxidative phosphorylation of ADP to ATP. Within the past decade, ATP synthase has been shown to play a role in cancer (165, 166) and was suggested as a promising therapeutic target, particularly when expressed on the cell surface (167).

Initially cell surface ATP synthase was discovered on endothelial cells as a target of angiostatin. Angiostatin was shown to inhibit tumor angiogenesis and thus it was evaluated as a therapeutic agent for various tumor cells. However, initial clinical trials were not as successful as expected, perhaps due to the lack of concomitant surrounding environment acidification and “undisturbed” highly efficient aerobic glycolysis (168).

Recently, cell surface ATP synthase was also discovered on some other tumor types and it has again been proposed as a direct target to treat tumor cells. Inhibition of cell surface ATP synthase with angiostatin, aurovertin, resveratrol and antibodies against α and β subunits of ATP synthase effectively and specifically killed corresponding cells, especially under low pH conditions. For cell types on which surface ATP synthase has been found, drugs that have been effectively used to target it and its possible functions have recently been reviewed

(169). This was achieved by recent discovery that an acidic microenvironment might trigger local destabilization of the extracellular matrix, facilitating tumor growth and tissue invasion (162, 170). Thus, due to the acidic microenvironment surrounding tumors, cell surface ATP synthase inhibition holds a new avenue to specifically kill tumor cells, either directly or by killing endothelial cells of the microvessels that nourish a tumor. Loss of ATP synthase expression would be expected to create a bottleneck in mitochondrial oxidative phosphorylation (162). Recently we have detected increased expression of ATP synthase β in SDHB- compared to VHL-derived primary tumors (Flidner et al. unpublished observation). Thus, the ATP synthase β subunit could represent a therapeutic target in patients with metastatic pheochromocytoma.

2.4. Pheochromocytoma and imaging flip-flop: A reflection of an oxidative stress and the Warburg effect

During oxidative stress tumor cells increase access to glucose to maintain a very high rate of glycolysis (the Warburg effect). This can be achieved either by an increased supply or, possibly more importantly, by an increased rate of glucose entry into tumor cells. This is accomplished by the overexpression of GLUT1 and four transporters that carry glucose into tumor cells (118). This unique phenomenon is the basis of the use of [^{18}F]-fluorodeoxyglycose positron emission tomography in cancer patients. This also applies to pheochromocytoma patients, especially those with *SDHB* mutations. Timmers et al. first observed that in patients with *SDHB* mutation [^{18}F]-fluorodeoxyglycose positron emission tomography is far superior to other functional imaging studies, even to the most specific radiopharmaceutical, [^{18}F]-fluorodopamine (171). This observation is also called the “flip-flop imaging phenomenon” and it has been used very successfully in the localization of SDHB-related tumors, especially metastatic disease.

2.4. Perspectives: Pheochromocytoma: future diagnostic and therapeutic approaches

Further elucidating the pathways of pheochromocytoma tumorigenesis will facilitate the search for new molecular and genetic markers for diagnosis and targets for treatment of malignant pheochromocytoma. Those markers can then be used for the development of new and improved treatments for metastatic pheochromocytoma. Microarray studies, genome-wide association studies, proteomics, and protein arrays are tools currently accessible to power this search. It remains to be established what the cut-off is for SDH enzyme activity to sufficiently prevent the development of pheochromocytoma and which succinate levels are required to translocate HIF-1 α into the nucleus to initiate tumorigenesis (172). If tumorigenesis is to be initiated, it remains to be answered whether only certain “pre-programmed” cells will “respond” to follow “the order” to become cancer cells.

Although HPLC with electrochemical detection provides the most commonly available measurement method for urinary fractionated metanephrines, newer methods involving mass spectrometry are likely to offer further improvement and replace HPLC in the near future. The analytical sensitivity required for measurement of low levels of free metanephrines in plasma remains to be determined; additionally, the widespread measurement of methoxytyramine remains to be introduced. Chromogranin A, another marker of neuroendocrine tumors including pheochromocytoma, may need to be re-evaluated in the diagnostic approach of patients with SDHB-related pheochromocytoma since it is widely accessible around the world. The search for new oxidative stress and glycolysis markers in plasma or urine using proteomics and metabolomic approaches represent additional diagnostic means, especially in those patients with SDHx- and VHL-related pheochromocytoma.

The introduction of a new imaging approach to detect impaired mitochondria would be an important achievement. The existence of possible correlation between the degree of impaired mitochondria and clinical outcome could serve as the basis for tailoring treatment approaches, including regular follow-ups.

The development of new therapeutic strategies based on what we have learned about oxidative and metabolic stress is another important task. Targeting the glycolytic pathway (e.g. using lactate dehydrogenase A inhibitors) could be an option; affecting the stabilization of HIF- α protein, chaperones, or co-chaperones to handle mutated proteins in a more efficient way could be another promising avenue of treatment. mTORC pathway inhibitors, especially combined ones (mTORC1 and mTORC2), could be preferred as they target both HIF-1 α and HIF-2 α resulting in their downregulation (**Table 4**). The iron responsive element of HIF-2 α binds iron regulatory protein 1 and this action results in the inhibition of HIF-2 α translation (122, 173). The introduction of new therapeutic molecules that would enhance the binding of iron regulatory protein 1 could provide a new venue to treat SDHB-related metastatic pheochromocytomas. The use of antioxidants (e.g. vitamin C and E, glutathione) is another option for to fight oxidative stress. Finally, IL-13R α 2, IGF1 receptor and insulin signaling pathways could become a valuable target for metastatic pheochromocytoma and paraganglioma therapy (174, 175).

Recently, treatment with the histone deacetylase (HDAC) inhibitors romidepsin and trichostatin A increased [^{123}I]-MIBG, [^{18}F]-fluorodopamine, and [^3H]-NE uptake in mouse pheochromocytoma cells *in vitro* and *in vivo* in liver metastatic lesions through the upregulation of the cell membrane norepinephrine transporter system. This approach may be used clinically to augment the therapeutic efficacy of [^{131}I]-MIBG in patients with advanced malignant pheochromocytoma and other related tumors such as neuroblastoma (176). It is yet to be established, how or to what degree HDAC inhibitors may affect oxidative phosphorylation and glycolysis in various tumors including pheochromocytoma (177).

In summary, various pheochromocytomas, especially those related to SDH gene mutations, can be viewed as an “oxidative stress disease” and could be successfully treated in the future by targeting oxidative phosphorylation, glycolysis, glucose sensing and its cell entry as well as microenvironment acidification and ATP handling. Furthermore, unraveling the abnormal function of various signaling pathways as a consequence of oxidative and metabolic stress will further help to treat these tumors with a better success.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, Eunice Kennedy Shriver NICHD. I thank Kathryn King and Victoria Martuzzi for their technical assistance.

References

1. Kohn A. Über die Nebenniere. *Prag Med Wschr.* 1898; 23:193–195.
2. Kohn A. Das chromaffine Gewebe. *Ergebn Anat Entwickl-Gesch.* 1902; 12:253–348.
3. Kohn A. Die Paraganglien. *Arch Mikr Anat.* 1903; 62:263–365.
4. Kohn A. Das chromaffine Gewebe. *Allg Wien Med Ztg.* 1903; 48:503–504.
5. Bock, P. The Paraganglia. Springer-Verlag; Berlin Heidelberg New York: 1982.
6. Pick L. Das Ganglioma embryonale sympathicum (Sympathoma embryonale), eine typisch bosartige Geschwulstform des sympathischen Nervensystems. *Klin Wochenschr.* 1912; 49:16–22.
7. Frankel F. Ein fall von doppelseitigem, völlig latent verlaufenden Nebennieren tumor und gleichzeitiger Nephritis mit Veränderungen am Circulationsapparat und Retinitis. *Virchows Arch.* 1886; 103:244–263.

8. Kelly HM, Piper MC, Wilder RE. Case of paroxysmal hypertension with paraganglioma of the right suprarenal gland. *Mayo Clin Proc.* 1936; 11:65–70.
9. Holton P. Noradrenaline in tumors of the adrenal medulla. *J Physiol.* 1949; 108:525–529.
10. Von Euler US. Increased urinary excretion of noradrenaline and adrenaline in cases of pheochromocytoma. *Ann Surg.* 1951; 134:929–933. [PubMed: 14895119]
11. Lund A, Moller K. [Noradrenalin in blood in pheochromocytoma.]. *Ugeskr Laeger.* 1951; 113:1068. [PubMed: 14867019]
12. Lund A. Adrenaline and noradrenaline in blood and urine in cases of pheochromocytoma. *Scand J Clin Lab Invest.* 1952; 4:263–265. [PubMed: 13048603]
13. Armstrong MD, Mc MA, Shaw KN. 3-Methoxy-4-hydroxy-D-mandelic acid, a urinary metabolite of norepinephrine. *Biochim Biophys Acta.* 1957; 25:422–423. [PubMed: 13471587]
14. Axelrod J. O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. *Science.* 1957; 126:400–401. [PubMed: 13467217]
15. Labrosse EH, Axelrod J, Kety SS. O-Methylation, the principal route of metabolism of epinephrine in man. *Science.* 1958; 128:593–594. [PubMed: 13580222]
16. Manger, W.; Gifford, R. Blackwell Science. Massachusetts; Cambridge: 1996. *Clinical and Experimental Pheochromocytoma.*
17. Manger WM, Gifford RW. Pheochromocytoma. *J Clin Hypertens.* 2002; 4:62–72.
18. Bravo EL, Tagle R. Pheochromocytoma: state-of-the-art and future prospects. *Endocr Rev.* 2003; 24:539–553. [PubMed: 12920154]
19. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T. Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res.* 2004; 27:193–202. [PubMed: 15080378]
20. Anderson GH Jr, Blakeman N, Streeten DH. The effect of age on prevalence of secondary forms of hypertension in 4429 consecutively referred patients. *J Hypertens.* 1994; 12:609–615. [PubMed: 7930562]
21. Epstein, FH.; Eckhoff, RD. The epidemiology of high blood pressure- geographic distributions and etiologic factors.. In: Stamler, J.; Stamler, R.; Pullman, TN., editors. *The epidemiology of hypertension.* Grune and Stratton; New York: 1967. p. 155-166.
22. Page LB. Epidemiologic evidence on the etiology of human hypertension and its possible prevention. *Am Heart J.* 1976; 91:527–534. [PubMed: 769520]
23. McBride W, Ferrario C, Lyle PA. Hypertension and medical informatics. *J Natl Med Assoc.* 2003; 95:1048–1056. [PubMed: 14651371]
24. Pacak, K.; Chrousos, GP.; Koch, CA.; Lenders, JW.; Eisenhofer, G. Pheochromocytoma: progress in diagnosis, therapy, and genetics.. In: Margioris, A.; Chrousos, GP., editors. *Adrenal Disorders.* 1 ed.. Humana Press; Totowa: 2001. p. 479-523.
25. Nagatsu T, Levitt M, Udenfriend S. Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. *J Biol Chem.* 1964; 239:2910–2917. [PubMed: 14216443]
26. Eisenhofer G, Aneman A, Friberg P, Hooper D, Fandriks L, Lonroth H, Hunyady B, Mezey E. Substantial production of dopamine in the human gastrointestinal tract. *J Clin Endocrinol Metab.* 1997; 82:3864–3871. [PubMed: 9360553]
27. Eisenhofer G, Aneman A, Hooper D, Holmes C, Goldstein DS, Friberg P. Production and metabolism of dopamine and norepinephrine in mesenteric organs and liver of swine. *Am J Physiol.* 1995; 268:G641–649. [PubMed: 7733289]
28. Eisenhofer G, Huynh TT, Hiroi M, Pacak K. Understanding catecholamine metabolism as a guide to the biochemical diagnosis of pheochromocytoma. *Rev Endocr Metab Disord.* 2001; 2:297–311. [PubMed: 11708294]
29. Eisenhofer G, Pecorella W, Pacak K, Hooper D, Kopin I, Goldstein D. The neuronal and extraneuronal origins of plasma 3-methoxy-4-hydroxyphenylglycol in rats. *J Auton Nerv Syst.* 1994; 50:93–107. [PubMed: 7844319]
30. Eisenhofer G, Rundqvist B, Aneman A, et al. Regional release and removal of catecholamines and extraneuronal metabolism to metanephrines. *J Clin Endocrinol Metab.* 1995; 80:3009–3017. [PubMed: 7559889]

31. Eisenhofer G, Aneman A, Hooper D, Rundqvist B, Friberg P. Mesenteric organ production, hepatic metabolism, and renal elimination of norepinephrine and its metabolites in humans. *J Neurochem.* 1996; 66:1565–1573. [PubMed: 8627312]
32. Ahlquist RP. A study of the adrenotropic receptors. *Am J Physiol.* 1948; 153:586–600. [PubMed: 18882199]
33. Lefkowitz RJ. Direct binding studies of adrenergic receptors: biochemical, physiologic, and clinical implications. *Ann Intern Med.* 1979; 91:450–458. [PubMed: 38720]
34. Hoffman BB, Lefkowitz RJ. Alpha-adrenergic receptor subtypes. *N Engl J Med.* 1980; 302:1390–1396. [PubMed: 6246426]
35. Motulsky HJ, Insel PA. Adrenergic receptors in man: direct identification, physiologic regulation, and clinical alterations. *N Engl J Med.* 1982; 307:18–29. [PubMed: 6123082]
36. Arch JR. The beta 3-adrenergic system and beta 3-adrenergic agonists. *Rev Endocr Metab Disord.* 2001; 2:385–393. [PubMed: 11725725]
37. Missale C, Fiorentini C, Collo G, Spano P. The neurobiology of dopamine receptors: evolution from the dual concept to heterodimer complexes. *J Recept Signal Transduct Res.* 2010; 30:347–354. [PubMed: 20684667]
38. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev.* 2011; 63:182–217. [PubMed: 21303898]
39. Pacak, K.; Keiser, H.; Eisenhofer, G. Pheochromocytoma.. In: De Groot, LS.; Jameson, JL., editors. *Textbook of Endocrinology.* 5th ed.. Elsevier Saunders; Philadelphia: 2005. p. 2501–2534.
40. Pacak K. Preoperative management of the pheochromocytoma patient. *J Clin Endocrinol Metab.* 2007; 92:4069–4079. [PubMed: 17989126]
41. Yamaguchi I, Kopin IJ. Differential inhibition of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses in pithed rats. *J Pharmacol Exp Ther.* 1980; 214:275–281. [PubMed: 6104724]
42. Langer SZ, Massingham R, Shepperson NB. Presence of postsynaptic alpha 2-adrenoreceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. *Clin Sci (Lond).* 1980; 59(Suppl 6):225s–228s. [PubMed: 6108815]
43. Eisenhofer G, Goldstein DS, Walther MM, Friberg P, Lenders JW, Keiser HR, Pacak K. Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. *J Clin Endocrinol Metab.* 2003; 88:2656–2666. [PubMed: 12788870]
44. Goldberg LI. The dopamine vascular receptor. *Biochem Pharmacol.* 1975; 24:651–653. [PubMed: 164871]
45. Creese I, Sibley DR, Leff SE. Agonist interactions with dopamine receptors: focus on radioligand-binding studies. *Fed Proc.* 1984; 43:2779–2784. [PubMed: 6383871]
46. Willems JL, Buylaert WA, Lefebvre RA, Bogaert MG. Neuronal dopamine receptors on autonomic ganglia and sympathetic nerves and dopamine receptors in the gastrointestinal system. *Pharmacol Rev.* 1985; 37:165–216. [PubMed: 2996038]
47. Himms-Hagen, J. Effects of catecholamines on metabolism.. In: Blaschko, H.; Muschoff, E., editors. *Catecholamines. Handbuch der experimentellen Pharmakologie.* Vol. 33. Springer-Verlag; Berlin: 1972. p. 363–462.
48. Ellis, S. Effects on the metabolism. In *Adrenergic Activators and Inhibitors.* In: Szekeres, L., editor. *Handbook of Experimental Pharmacology.* Vol. 54. Springer-Verlag; Berlin: 1980. p. 319–349.
49. Goldenberg M, Pines KL, Baldwin ED. The Hemodynamic response of man to norepinephrine and epinephrine and its relation to the problem of hypertension. *Am J Med.* 1948; 5:792–806. [PubMed: 18100389]
50. Goldenberg M, Aranow HJ, Smith AA. Pheochromocytoma and essential hypertensive vascular disease. *Arch Intern Med.* 1950; 86:823–836.
51. Bravo EL, Tarazi RC, Fouad FM, Vidt DG, Gifford RW Jr. Clonidine-suppression test: a useful aid in the diagnosis of pheochromocytoma. *N Engl J Med.* 1981; 305:623–626. [PubMed: 7266587]
52. Bravo EL, Gifford RW Jr. Pheochromocytoma. *Endocrinol Metab Clin North Am.* 1993; 22:329–341. [PubMed: 8325290]

53. Tsujimoto G, Honda K, Hoffman BB, Hashimoto K. Desensitization of postjunctional alpha 1- and alpha 2-adrenergic receptor-mediated vasopressor responses in rat harboring pheochromocytoma. *Circ Res.* 1987; 61:86–98. [PubMed: 3038368]
54. Crout JR, Sjoerdsma A. Turnover and Metabolism of Catecholamines in Patients with Pheochromocytoma. *J Clin Invest.* 1964; 43:94–102. [PubMed: 14105236]
55. Feldman JM. Phenylethanolamine-N-methyltransferase activity determines the epinephrine concentration of pheochromocytomas. *Res Commun Chem Pathol Pharmacol.* 1981; 34:389–398. [PubMed: 7323441]
56. Osamura RY, Onoda N. Immunohistochemical localization of various peptides in the infundibular nuclei of human adult hypothalamus. *Tokai J Exp Clin Med.* 1988; 13:71–78. [PubMed: 3074524]
57. Kimura N, Miura Y, Nagatsu I, Nagura H. Catecholamine synthesizing enzymes in 70 cases of functioning and non-functioning pheochromocytoma and extra-adrenal paraganglioma. *Virchows Arch A Pathol Anat Histopathol.* 1992; 421:25–32. [PubMed: 1353277]
58. Tümer N, Brown JW, Carballeira A, Fishman LM. Tyrosine hydroxylase gene expression in varying forms of human pheochromocytoma. *Life Sci.* 1996; 59:1659–1665. [PubMed: 8913331]
59. Eisenhofer G, Huynh TT, Elkahoul A, Morris JC, Bratslavsky G, Linehan WM, Zhuang Z, Balgley BM, Lee CS, Mannelli M, Lenders JW, Bornstein SR, Pacak K. Differential expression of the regulated catecholamine secretory pathway in different hereditary forms of pheochromocytoma. *Am J Physiol Endocrinol Metab.* 2008; 295:E1223–1233. [PubMed: 18854424]
60. Timmers HJ, Pacak K, Huynh TT, Abu-Asab M, Tsokos M, Merino MJ, Baysal BE, Adams KT, Eisenhofer G. Biochemically silent abdominal paragangliomas in patients with mutations in the succinate dehydrogenase subunit B gene. *J Clin Endocrinol Metab.* 2008; 93:4826–4832. [PubMed: 18840642]
61. Eisenhofer G, Goldstein DS, Sullivan P, Csako G, Brouwers FM, Lai EW, Adams KT, Pacak K. Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. *J Clin Endocrinol Metab.* 2005; 90:2086–2075.
62. Nagatsu T, Mizutani K, Sudo Y, Nagatsu I. Tyrosine hydroxylase in human adrenal glands and human pheochromocytoma. *Clin Chim Acta.* 1972; 39:417–424. [PubMed: 4402930]
63. Jarrott B, Louis WJ. Abnormalities in enzymes involved in catecholamine synthesis and catabolism in pheochromocytoma. *Clin Sci Mol Med.* 1977; 53:529–535. [PubMed: 22422]
64. Nakada T, Furuta H, Katayama T. Catecholamine metabolism in pheochromocytoma and normal adrenal medullae. *J Urol.* 1988; 140:1348–1351. [PubMed: 2903937]
65. Eisenhofer G, Lenders JW, Timmers H, Mannelli M, Grebe SK, Hofbauer LC, Bornstein SR, Tiebel O, Adams K, Bratslavsky G, Linehan WM, Pacak K. Measurements of Plasma Methoxytyramine, Normetanephrine, and Metanephrine as Discriminators of Different Hereditary Forms of Pheochromocytoma. *Clin Chem.* 2011; 57:411–420. [PubMed: 21262951]
66. Rao F, Keiser HR, O'Connor DT. Malignant pheochromocytoma. Chromaffin granule transmitters and response to treatment. *Hypertension.* 2000; 36:1045–1052. [PubMed: 11116123]
67. Schlumberger M, Gicquel C, Lumbroso J, Tenenbaum F, Comoy E, Bosq J, Fonseca E, Ghillani PP, Aubert B, Travagli JP. Malignant pheochromocytoma: clinical, biological, histologic and therapeutic data in a series of 20 patients with distant metastases. *J Endocrinol Invest.* 1992; 15:631–642. [PubMed: 1479146]
68. Proye C, Fossati P, Fontaine P, Lefebvre J, Decoulx M, Wemeau JL, Dewailly D, Rwamasirabo E, Cecat P. Dopamine-secreting pheochromocytoma: an unrecognized entity? Classification of pheochromocytomas according to their type of secretion. *Surgery.* 1986; 100:1154–1162. [PubMed: 3787474]
69. Anton AH, Greer M, Sayre DF, Williams CM. Dihydroxyphenylalanine secretion in a malignant pheochromocytoma. *Am J Med.* 1967; 42:469–475. [PubMed: 6018863]
70. Goldstein DS, Stull R, Eisenhofer G, Sisson JC, Weder A, Averbuch SD, Keiser HR. Plasma 3,4-dihydroxyphenylalanine (dopa) and catecholamines in neuroblastoma or pheochromocytoma. *Ann Intern Med.* 1986; 105:887–888. [PubMed: 3096183]

71. McClean DR, Sinclair LM, Yandle TG, Nicholls MG. Malignant pheochromocytoma with high circulating DOPA, and clonidine- suppressible noradrenaline. *Blood Press.* 1995; 4:215–217. [PubMed: 7496559]
72. John H, Ziegler WH, Hauri D, Jaeger P. Pheochromocytomas: can malignant potential be predicted? *Urology.* 1999; 53:679–683. [PubMed: 10197840]
73. Eisenhofer G, Pacak K, Huynh TT, Qin N, Bratslavsky G, Linehan WM, Mannelli M, Friberg P, Grebe SK, Timmers HJ, Bornstein SR, Lenders JW. Catecholamine metabolomic and secretory phenotypes in pheochromocytoma. *Endocr Relat Cancer.* 2011; 18:97–111. [PubMed: 21051559]
74. Wurtman RJ, Axelrod J. Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J Biol Chem.* 1966; 241:2301–2305. [PubMed: 4287854]
75. Wong DL, Siddall BJ, Ebert SN, Bell RA, Her S. Phenylethanolamine N-methyltransferase gene expression: synergistic activation by Egr-1, AP-2 and the glucocorticoid receptor. *Brain Res Mol Brain Res.* 1998; 61:154–161. [PubMed: 9795195]
76. Rosas AL, Kasperlik-Zaluska AA, Papierska L, Bass BL, Pacak K, Eisenhofer G. Pheochromocytoma crisis induced by glucocorticoids: a report of four cases and review of the literature. *Eur J Endocrinol.* 2008; 158:423–429. [PubMed: 18299478]
77. Pacak K, Linehan WM, Eisenhofer G, Walther MM, Goldstein DS. Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. *Ann Intern Med.* 2001; 134:315–329. [PubMed: 11182843]
78. Brouwers FM, Lenders JW, Eisenhofer G, Pacak K. Pheochromocytoma as an endocrine emergency. *Rev Endocr Metab Disord.* 2003; 4:121–128. [PubMed: 12766539]
79. Huynh TT, Pacak K, Brouwers FM, Abu-Asab MS, Worrell RA, Walther MM, Elkahloun AG, Goldstein DS, Cleary S, Eisenhofer G. Different expression of catecholamine transporters in pheochromocytomas from patients with von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2. *Eur J Endocrinol.* 2005; 153:551–563. [PubMed: 16189177]
80. Schuttler J, Westhofen P, Kania U, Ihmsen H, Kammerecker S, Hirner A. Quantitative assessment of catecholamine secretion as a rational principle of anesthesia management in pheochromocytoma surgery. *Anesthesiol Intensivmed Notfallmed Schmerzther.* 1995; 30:341–349. [PubMed: 8541435]
81. Pacak K, Fojo T, Goldstein DS, Eisenhofer G, Walther MM, Linehan WM, Bachenheimer L, Abraham J, Wood BJ. Radiofrequency ablation: a novel approach for treatment of metastatic pheochromocytoma. *J Natl Cancer Inst.* 2001; 93:648–649. [PubMed: 11309443]
82. Brouwers FM, Eisenhofer G, Lenders JW, Pacak K. Emergencies caused by pheochromocytoma, neuroblastoma, or ganglioneuroma. *Endocrinol Metab Clin North Am.* 2006; 35:699–724. [PubMed: 17127142]
83. Bravo E. Evolving concepts in the pathophysiology, diagnosis, and treatment of pheochromocytoma. *Endocr Rev.* 1994; 15:356–368. [PubMed: 8076587]
84. Shawar L, Svec F. Pheochromocytoma with elevated metanephrines as the only biochemical finding. *J La State Med Soc.* 1996; 148:535–538. [PubMed: 8990798]
85. Eisenhofer G, Keiser H, Friberg P, Mezey E, Huynh TT, Hiremagalur B, Ellingson T, Duddempudi S, Eijssbouts A, Lenders JW. Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors. *J Clin Endocrinol Metab.* 1998; 83:2175–2185. [PubMed: 9626157]
86. Shen SJ, Cheng HM, Chiu AW, Chou CW, Chen JY. Perioperative hypertensive crisis in clinically silent pheochromocytomas: report of four cases. *Chang Gung Med J.* 2005; 28:44–50. [PubMed: 15804148]
87. Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G. Biochemical diagnosis of pheochromocytoma: which test is best? *JAMA.* 2002; 287:1427–1434. [PubMed: 11903030]
88. Raber W, Raffesberg W, Bischof M, Scheuba C, Niederle B, Gasic S, Waldhausl W, Roden M. Diagnostic efficacy of unconjugated plasma metanephrines for the detection of pheochromocytoma. *Arch Intern Med.* 2000; 160:2957–2963. [PubMed: 11041903]

89. Unger N, Pitt C, Schmidt IL, Walz MK, Schmid KW, Philipp T, Mann K, Petersenn S. Diagnostic value of various biochemical parameters for the diagnosis of pheochromocytoma in patients with adrenal mass. *Eur J Endocrinol*. 2006; 154:409–417. [PubMed: 16498054]
90. Sawka AM, Gafni A, Thabane L, Young WF Jr. The economic implications of three biochemical screening algorithms for pheochromocytoma. *J Clin Endocrinol Metab*. 2004; 89:2859–2866. [PubMed: 15181069]
91. Gardet V, Gatta B, Simonnet G, Tabarin A, Chene G, Ducassou D, Corcuff JB. Lessons from an unpleasant surprise: a biochemical strategy for the diagnosis of pheochromocytoma. *J Hypertens*. 2001; 19:1029–1035. [PubMed: 11403350]
92. Gerlo E, Sevens C. Urinary and plasma catecholamines and urinary catecholamine metabolites in pheochromocytoma: diagnostic value in 19 cases. *Clin Chem*. 1994; 40:250–256. [PubMed: 7906208]
93. Guller U, Turek J, Eubanks S, DeLong ER, Oertli D, Feldman JM. Detecting pheochromocytoma: defining the most sensitive test. *Ann Surg*. 2006; 243:102–107. [PubMed: 16371743]
94. Davidson DF. Pheochromocytoma with normal urinary catecholamines: the potential value of urinary free metadrenalines. *Ann Clin Biochem*. 2002; 39:557–566. [PubMed: 12564837]
95. Grossman A, Pacak K, Sawka A, Lenders JW, Harlander D, Peaston RT, Reznick R, Sisson J, Eisenhofer G. Biochemical diagnosis and localization of pheochromocytoma: can we reach a consensus? *Ann N Y Acad Sci*. 2006; 1073:332–347. [PubMed: 17102103]
96. Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Pheochromocytoma. *Lancet*. 2005; 366:665–675. [PubMed: 16112304]
97. Elliott WJ, Murphy MB. Reduced specificity of the clonidine suppression test in patients with normal plasma catecholamine levels. *Am J Med*. 1988; 84:419–424. [PubMed: 3348244]
98. Grossman E, Goldstein D, Hoffman A, Keiser H. Glucagon and clonidine testing in the diagnosis of pheochromocytoma. *Hypertension*. 1991; 17:733–741. [PubMed: 2045133]
99. Taylor HC, Mayes D, Anton AH. Clonidine suppression test for pheochromocytoma: examples of misleading results. *J Clin Endocrinol Metab*. 1986; 63:238–242. [PubMed: 3711261]
100. Lenders JW, Pacak K, Huynh TT, Sharabi Y, Mannelli M, Bratslavsky G, Goldstein DS, Bornstein SR, Eisenhofer G. Low sensitivity of glucagon provocative testing for diagnosis of pheochromocytoma. *J Clin Endocrinol Metab*. 2010; 95:238–245. [PubMed: 19897672]
101. Seyfried TN, Shelton LM. Cancer as a metabolic disease. *Nutr Metab (Lond)*. 2010; 7:7. [PubMed: 20181022]
102. Mathupala SP, Ko YH, Pedersen PL. The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies. *Biochim Biophys Acta*. 2010; 1797:1225–1230. [PubMed: 20381449]
103. Warburg O. On the origin of cancer cells. *Science*. 1956; 123:309–314. [PubMed: 13298683]
104. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer*. 2005; 5:857–866. [PubMed: 16327764]
105. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med*. 1991; 91:31S–38S. [PubMed: 1928209]
106. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000; 408:239–247. [PubMed: 11089981]
107. Spitz DR, Sim JE, Ridnour LA, Galoforo SS, Lee YJ. Glucose deprivation-induced oxidative stress in human tumor cells. A fundamental defect in metabolism? *Ann N Y Acad Sci*. 2000; 899:349–362. [PubMed: 10863552]
108. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. *Nat Rev Cancer*. 2003; 3:193–202. [PubMed: 12612654]
109. Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, Jouanno E, Jeunemaitre X, Benit P, Tzagoloff A, Rustin P, Bertherat J, Favier J, Gimenez-Roqueplo AP. SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet*. 2010; 19:3011–3020. [PubMed: 20484225]
110. Hao HX, Khalimonchuk O, Schradars M, Dephoure N, Bayley JP, Kunst H, Devilee P, Cremers CW, Schiffman JD, Bentz BG, Gygi SP, Winge DR, Kremer H, Rutter J. SDH5, a gene required

- for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science*. 2009; 325:1139–1142. [PubMed: 19628817]
111. Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol*. 2008; 28:718–731. [PubMed: 17967865]
  112. Knudson AJ, Strong L. Mutation and cancer: neuroblastoma and pheochromocytoma. *Am J Hum Genet*. 1972; 24:514–532. [PubMed: 4340974]
  113. Favier J, Gimenez-Roqueplo AP. Pheochromocytomas: The (pseudo)-hypoxia hypothesis. *Best Pract Res Clin Endocrinol Metab*. 2010; 24:957–968. [PubMed: 21115164]
  114. Smith EH, Janknecht R, Maher LJ 3rd. Succinate inhibition of alpha-ketoglutarate-dependent enzymes in a yeast model of paraganglioma. *Hum Mol Genet*. 2007; 16:3136–3148. [PubMed: 17884808]
  115. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature*. 1998; 394:694–697. [PubMed: 9716135]
  116. Lee S, Nakamura E, Yang H, Wei W, Linggi MS, Sajan MP, Farese RV, Freeman RS, Carter BD, Kaelin WG Jr, Schlisio S. Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer Cell*. 2005; 8:155–167. [PubMed: 16098468]
  117. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell*. 2005; 7:77–85. [PubMed: 15652751]
  118. Levine AJ, Puzio-Kuter AM. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science*. 2010; 330:1340–1344. [PubMed: 21127244]
  119. Favier J, Briere JJ, Burnichon N, Riviere J, Vescovo L, Benit P, Giscos-Douriez I, De Reynies A, Bertherat J, Badoual C, Tissier F, Amar L, Libe R, Plouin PF, Jeunemaitre X, Rustin P, Gimenez-Roqueplo AP. The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS One*. 2009; 4:e7094. [PubMed: 19763184]
  120. Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, Kung AL, Sanso G, Powers JF, Tischler AS, Hodin R, Heitritter S, Moore F, Dluhy R, Sosa JA, Ocal IT, Benn DE, Marsh DJ, Robinson BG, Schneider K, Garber J, Arum SM, Korbonits M, Grossman A, Pigny P, Toledo SP, Nose V, Li C, Stiles CD. A HIF1 α regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS Genet*. 2005; 1:72–80. [PubMed: 16103922]
  121. Pollard PJ, El-Bahrawy M, Poulson R, Elia G, Killick P, Kelly G, Hunt T, Jeffery R, Seedhar P, Barwell J, Latif F, Gleeson MJ, Hodgson SV, Stamp GW, Tomlinson IP, Maher ER. Expression of HIF-1 α , HIF-2 α (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. *J Clin Endocrinol Metab*. 2006; 91:4593–4598. [PubMed: 16954163]
  122. Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. *Nat Rev Urol*. 2010; 7:277–285. [PubMed: 20448661]
  123. Cervera AM, Apostolova N, Crespo FL, Mata M, McCreath KJ. Cells silenced for SDHB expression display characteristic features of the tumor phenotype. *Cancer Res*. 2008; 68:4058–4067. [PubMed: 18519664]
  124. Arias-Stella J, Valcarcel J. The human carotid body at high altitudes. *Pathol Microbiol (Basel)*. 1973; 39:292–297.
  125. Arias-Stella J. The carotid body at high altitude. *Meet Am Ass Pathol Bact*. 1969 Abstract 150.
  126. Arias-Stella J, Bustos F. Chronic hypoxia and chemodectomas in bovines at high altitudes. *Arch Pathol Lab Med*. 1976; 100:636–639. [PubMed: 187143]
  127. Edwards C, Heath D, Harris P, Castillo Y, Kruger H, Arias-Stella J. The carotid body in animals at high altitude. *J Pathol*. 1971; 104:231–238. [PubMed: 5135659]
  128. Cornog JL, Wilkinson JH, Arvan DA, Freed RM, Sellers AM, Barker C. Extra-adrenal pheochromocytoma. Some electron microscopic and biochemical studies. *Am J Med*. 1970; 48:654–660. [PubMed: 5425242]

129. Cerecer-Gil NY, Figuera LE, Llamas FJ, Lara M, Escamilla JG, Ramos R, Estrada G, Hussain AK, Gaal J, Korpershoek E, de Krijger RR, Dinjens WN, Devilee P, Bayley JP. Mutation of SDHB is a cause of hypoxia-related high-altitude paraganglioma. *Clin Cancer Res*. 2010; 16:4148–4154. [PubMed: 20592014]
130. Housley SL, Lindsay RS, Young B, McConachie M, Mechan D, Baty D, Christie L, Rahilly M, Qureshi K, Fleming S. Renal carcinoma with giant mitochondria associated with germ-line mutation and somatic loss of the succinate dehydrogenase B gene. *Histopathology*. 2010; 56:405–408. [PubMed: 20459544]
131. Watanabe H, Burnstock G, Jarrott B, Louis WJ. Mitochondrial abnormalities in human pheochromocytoma. *Cell Tissue Res*. 1976; 172:281–288. [PubMed: 991214]
132. Heutink P, van der Mey AG, Sandkuijl LA, van Gils AP, Bardoel A, Breedveld GJ, van Vliet M, van Ommen GJ, Cornelisse CJ, Oostra BA, et al. A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. *Hum Mol Genet*. 1992; 1:7–10. [PubMed: 1301144]
133. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW 3rd, Cornelisse CJ, Devilee P, Devlin B. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science*. 2000; 287:848–851. [PubMed: 10657297]
134. King A, Selak MA, Gottlieb E. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. *Oncogene*. 2006; 25:4675–4682. [PubMed: 16892081]
135. Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de Paillerets B, Chabre O, Chamontin B, Delemer B, Giraud S, Murat A, Niccoli-Sire P, Richard S, Rohmer V, Sadoul JL, Stropf L, Schlumberger M, Bertagna X, Plouin PF, Jeunemaitre X, Gimenez-Roqueplo AP. Genetic testing in pheochromocytoma or functional paraganglioma. *J Clin Oncol*. 2005; 23:8812–8818. [PubMed: 16314641]
136. Benn DE, Gimenez-Roqueplo AP, Reilly JR, Bertherat J, Burgess J, Byth K, Croxson M, Dahia PL, Elston M, Gimm O, Henley D, Herman P, Murday V, Niccoli-Sire P, Pasiaka JL, Rohmer V, Tucker K, Jeunemaitre X, Marsh DJ, Plouin PF, Robinson BG. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. *J Clin Endocrinol Metab*. 2006; 91:827–836. [PubMed: 16317055]
137. Brouwers FM, Eisenhofer G, Tao JJ, Kant JA, Adams KT, Linehan WM, Pacak K. High Frequency of SDHB Germline Mutations in Patients with Malignant Catecholamine-Producing Paragangliomas: Implications for Genetic Testing. *J Clin Endocrinol Metab*. 2006; 91:4505–4509. [PubMed: 16912137]
138. Timmers HJ, Kozupa A, Eisenhofer G, Raygada M, Adams KT, Solis D, Lenders JW, Pacak K. Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab*. 2007; 92:779–786. [PubMed: 17200167]
139. Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA*. 2004; 292:943–951. [PubMed: 15328326]
140. Pham TH, Moir C, Thompson GB, Zarroug AE, Hamner CE, Farley D, van Heerden J, Lteif AN, Young WF Jr. Pheochromocytoma and paraganglioma in children: a review of medical and surgical management at a tertiary care center. *Pediatrics*. 2006; 118:1109–1117. [PubMed: 16951005]
141. Prodanov T, Havekes B, Nathanson KL, Adams KT, Pacak K. Malignant paraganglioma associated with succinate dehydrogenase subunit B in an 8-year-old child: the age of first screening? *Pediatr Nephrol*. 2009; 24:1239–1242. [PubMed: 19189136]
142. Khorram-Manesh A, Ahlman H, Nilsson O, Friberg P, Oden A, Stenstrom G, Hansson G, Stenquist O, Wangberg B, Tisell LE, Jansson S. Long-term outcome of a large series of patients surgically treated for pheochromocytoma. *J Intern Med*. 2005; 258:55–66. [PubMed: 15953133]
143. Plouin PF, Chatellier G, Fofol I, Corvol P. Tumor recurrence and hypertension persistence after successful pheochromocytoma operation. *Hypertension*. 1997; 29:1133–1139. [PubMed: 9149678]

144. Allibhai Z, Rodrigues G, Brecevic E, Neumann HP, Winkquist E. Malignant pheochromocytoma associated with germline mutation of the SDHB gene. *J Urol*. 2004; 172:1409–1410. [PubMed: 15371856]
145. Neumann HPH, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, Schipper J, Klisch J, Althoefer C, Zerres K, Januszewicz A, Eng C. Germ-line mutations in nonsyndromic pheochromocytoma. *New Engl J Med*. 2002; 346:1459–1466. [PubMed: 12000816]
146. Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F, Maher ER. Germline SDHB mutations and familial renal cell carcinoma. *J Natl Cancer Inst*. 2008; 100:1260–1262. [PubMed: 18728283]
147. Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peczkowska M, Morrison CD, Lehtonen R, Januszewicz A, Jarvinen H, Juhola M, Mecklin JP, Pukkala E, Herva R, Kiuru M, Nupponen NN, Aaltonen LA, Neumann HP, Eng C. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet*. 2004; 74:153–159. [PubMed: 14685938]
148. Solis DC, Burnichon N, Timmers HJ, Raygada MJ, Kozupa A, Merino MJ, Makey D, Adams KT, Venisse A, Gimenez-Roqueplo AP, Pacak K. Penetrance and clinical consequences of a gross SDHB deletion in a large family. *Clin Genet*. 2009; 75:354–363. [PubMed: 19389109]
149. Baysal BE, Willett-Brozick JE, Lawrence EC, Drowdlic CM, Savul SA, McLeod DR, Yee HA, Brackmann DE, Slattery WH 3rd, Myers EN, Ferrell RE, Rubinstein WS. Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. *J Med Genet*. 2002; 39:178–183. [PubMed: 11897817]
150. Baysal BE. On the association of succinate dehydrogenase mutations with hereditary paraganglioma. *Trends Endocrinol Metab*. 2003; 14:453–459. [PubMed: 14643060]
151. van Duinen N, Steenvoorden D, Kema IP, Jansen JC, Vriends AH, Bayley JP, Smit JW, Romijn JA, Corssmit EP. Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. *J Clin Endocrinol Metab*. 2010; 95:209–214. [PubMed: 19897674]
152. Ghayee HK, Havekes B, Corssmit EP, Eisenhofer G, Hammes SR, Ahmad Z, Tessnow A, Lazurova I, Adams KT, Fojo AT, Pacak K, Auchus RJ. Mediastinal paragangliomas: association with mutations in the succinate dehydrogenase genes and aggressive behavior. *Endocr Relat Cancer*. 2009; 16:291–299. [PubMed: 19075037]
153. King KS, Whatley MA, Alexopoulos DK, Reynolds JC, Chen CC, Mattox DE, Jacobs S, Pacak K. The use of functional imaging in a patient with head and neck paragangliomas. *J Clin Endocrinol Metab*. 2010; 95:481–482. [PubMed: 20133468]
154. Hensen EF, van Duinen N, Jansen JC, Corssmit EP, Tops CM, Romijn JA, Vriends AH, van der Mey AG, Cornelisse CJ, Devilee P, Bayley JP. High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands. *Clin Genet*. 2011 in press.
155. Mannelli M, Ercolino T, Giache V, Simi L, Cirami C, Parenti G. Genetic screening for pheochromocytoma: should SDHC gene analysis be included? *J Med Genet*. 2007; 44:586–587. [PubMed: 17557926]
156. Opocher G, Schiavi F. Genetics of pheochromocytomas and paragangliomas. *Best Pract Res Clin Endocrinol Metab*. 2010; 24:943–956. [PubMed: 21115163]
157. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet*. 2000; 26:268–270. [PubMed: 11062460]
158. Schiavi F, Boedeker CC, Bausch B, Peczkowska M, Gomez CF, Strassburg T, Pawlu C, Buchta M, Salzmann M, Hoffmann MM, Berlis A, Brink I, Cybulla M, Muresan M, Walter MA, Forrer F, Valimaki M, Kawecki A, Szutkowski Z, Schipper J, Walz MK, Pigny P, Bauters C, Willett-Brozick JE, Baysal BE, Januszewicz A, Eng C, Opocher G, Neumann HP. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. *JAMA*. 2005; 294:2057–2063. [PubMed: 16249420]
159. Gaal J, Burnichon N, Korpershoek E, Roncelin I, Bertherat J, Plouin PF, de Krijger RR, Gimenez-Roqueplo AP, Dinjens WN. Isocitrate dehydrogenase mutations are rare in pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab*. 2010; 95:1274–1278. [PubMed: 19915015]

160. Isidoro A, Martinez M, Fernandez PL, Ortega AD, Santamaria G, Chamorro M, Reed JC, Cuezva JM. Alteration of the bioenergetic phenotype of mitochondria is a hallmark of breast, gastric, lung and oesophageal cancer. *Biochem J*. 2004; 378:17–20. [PubMed: 14683524]
161. Chang HJ, Lee MR, Hong SH, Yoo BC, Shin YK, Jeong JY, Lim SB, Choi HS, Jeong SY, Park JG. Identification of mitochondrial FoF1-ATP synthase involved in liver metastasis of colorectal cancer. *Cancer Sci*. 2007; 98:1184–1191. [PubMed: 17559425]
162. Cuezva JM, Krajewska M, de Heredia ML, Krajewski S, Santamaria G, Kim H, Zapata JM, Marusawa H, Chamorro M, Reed JC. The bioenergetic signature of cancer: a marker of tumor progression. *Cancer Res*. 2002; 62:6674–6681. [PubMed: 12438266]
163. Moser TL, Stack MS, Asplin I, Enghild JJ, Hojrup P, Everitt L, Hubchak S, Schnaper HW, Pizzo SV. Angiostatin binds ATP synthase on the surface of human endothelial cells. *Proc Natl Acad Sci U S A*. 1999; 96:2811–2816. [PubMed: 10077593]
164. Yonally SK, Capaldi RA. The F(1)F(0) ATP synthase and mitochondrial respiratory chain complexes are present on the plasma membrane of an osteosarcoma cell line: An immunocytochemical study. *Mitochondrion*. 2006; 6:305–314. [PubMed: 17113362]
165. Willers IM, Cuezva JM. Post-transcriptional regulation of the mitochondrial H(+)-ATP synthase: A key regulator of the metabolic phenotype in cancer. *Biochim Biophys Acta*. 2010 in press.
166. Shin YK, Yoo BC, Chang HJ, Jeon E, Hong SH, Jung MS, Lim SJ, Park JG. Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance. *Cancer Res*. 2005; 65:3162–3170. [PubMed: 15833846]
167. Pedersen PL. The cancer cell's "power plants" as promising therapeutic targets: an overview. *J Bioenerg Biomembr*. 2007; 39:1–12. [PubMed: 17404823]
168. Beerepoot LV, Witteveen EO, Groenewegen G, Fogler WE, Sim BK, Sidor C, Zonnenberg BA, Schramel F, Gebbink MF, Voest EE. Recombinant human angiostatin by twice-daily subcutaneous injection in advanced cancer: a pharmacokinetic and long-term safety study. *Clin Cancer Res*. 2003; 9:4025–4033. [PubMed: 14519623]
169. Vantourout P, Radojkovic C, Lichtenstein L, Pons V, Champagne E, Martinez LO. Ecto-F-ATPase: a moonlighting protein complex and an unexpected apoA-I receptor. *World J Gastroenterol*. 2010; 16:5925–5935. [PubMed: 21157968]
170. Harguindey S, Arranz JL, Wahl ML, Orive G, Reshkin SJ. Proton transport inhibitors as potentially selective anticancer drugs. *Anticancer Res*. 2009; 29:2127–2136. [PubMed: 19528473]
171. Timmers HJ, Kozupa A, Chen CC, Carrasquillo JA, Ling A, Eisenhofer G, Adams KT, Solis D, Lenders JW, Pacak K. Superiority of fluorodeoxyglucose positron emission tomography to other functional imaging techniques in the evaluation of metastatic SDHB-associated pheochromocytoma and paraganglioma. *J Clin Oncol*. 2007; 25:2262–2269. [PubMed: 17538171]
172. Briere JJ, Favier J, Benit P, El Ghouzzi V, Lorenzato A, Rabier D, Di Renzo MF, Gimenez-Roqueplo AP, Rustin P. Mitochondrial succinate is instrumental for HIF1alpha nuclear translocation in SDHA-mutant fibroblasts under normoxic conditions. *Hum Mol Genet*. 2005; 14:3263–3269. [PubMed: 16195397]
173. Chen G, Fillebeen C, Wang J, Pantopoulos K. Overexpression of iron regulatory protein 1 suppresses growth of tumor xenografts. *Carcinogenesis*. 2007; 28:785–791. [PubMed: 17127713]
174. Lai EW, Joshi BH, Martiniova L, Dogra R, Fujisawa T, Leland P, de Krijger RR, Lubensky IA, Elkahoul AG, Morris JC, Puri RK, Pacak K. Overexpression of interleukin-13 receptor-alpha2 in neuroendocrine malignant pheochromocytoma: a novel target for receptor directed anti-cancer therapy. *J Clin Endocrinol Metab*. 2009; 94:2952–2957. [PubMed: 19491224]
175. Heidegger I, Pircher A, Klocker H, Massoner P. Targeting the insulin-like growth factor network in cancer therapy. *Cancer Biol Ther*. 2011 in press.
176. Martiniova L, Perera SM, Brouwers FM, Alesci S, Abu-Asab M, Marvelle AF, Kiesewetter DO, Thomasson D, Morris JC, Kvetnansky R, Tischler AS, Reynolds JC, Fojo AT, Pacak K. Increased uptake of [123I]meta-iodobenzylguanidine, [18F]fluorodopamine, and [3H]norepinephrine in mouse pheochromocytoma cells and tumors after treatment with the histone deacetylase inhibitors. *Endocr Relat Cancer*. 2011; 18:143–157. [PubMed: 21098082]

177. Alcarraz-Vizan G, Boren J, Lee WN, Cascante M. Histone deacetylase inhibition results in a common metabolic profile associated with HT29 differentiation. *Metabolomics*. 2010; 6:229–237. [PubMed: 20445757]
178. Kantorovich V, Pacak K. Pheochromocytoma and paraganglioma. *Prog Brain Res*. 2010; 182:343–373. [PubMed: 20541673]
179. Eisenhofer G, Lenders J, Linehan W, Walther M, Goldstein D, Keiser H. Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. *N Engl J Med*. 1999; 340:1872–1879. [PubMed: 10369850]
180. Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E, Michaud M, Zischka H, Castedo M, Kroemer G. Mitochondrial gateways to cancer. *Mol Aspects Med*. 2010; 31:1–20. [PubMed: 19698742]

To make a great dream come true, the first requirement is a great capacity to dream; the second is persistence – a faith in the dream.

Hans Selye

Figure 1.

The catecholamine biosynthetic pathway in a chromaffin or pheochromocytoma cell. Note NE and EPI accumulated in storage vesicles are ready for immediate release into bloodstream. Abbreviations: DOPA: Dihydroxyphenylalanine; PNMT: phenylethanolamine N-methyltransferase. Adapted from Kantorovich et al. (178).

Figure 2.

The main pathway for metabolism of NE and EPI derived from adrenal medullary and sympathoneuronal sources. Deamination (the major intraneuronal pathway of catecholamine metabolism using MOA), O-methylation by COMT, or sulfate conjugation produce various metabolites and conjugates from which plasma free metanephrines are currently the most commonly used in the biochemical diagnosis of pheochromocytoma (179). Abbreviations: NE: norepinephrine; EPI: epinephrine; MN: metanephrine; NMN: normetanephrine; DHPG: 3,4-dihydroxyphenylglycol; MHPG: 3-methoxy-4-hydroxyphenylglycol; VMA: vanillylmandelic acid, SO₄: sulfate conjugates; SULT1A3: phenolsulfotransferase type 1A3.

Constitutive Secretory Pathway

**Spontaneous – Ca^{2+} -independent –
Rapid – Continuous –
Not responsive to secretagogues**

Regulated Secretory Pathway

**Ca^{2+} -dependent – Storage pool of vesicles held in cytoskeletal frame
Large dense core vesicles –
Vesicle sorting – Peptide and protein packaging**

Figure 3.

A schematic model showing the existence of distinct secretory pathways in pheochromocytoma. Insets indicate magnifications of secretory granules to illustrate the catecholamine biosynthetic pathways and catecholamine regulated secretory pathways where annexin A7, rabphilin 3A, synaptotagmin play an important role. Continuous secretory pathways is Ca^{2+} -independent, rapid, and continuous; regulated secretory pathway is Ca^{2+} - and a storage pool-dependent and intermittent. Abbreviations: TYR, tyrosine; DOPA, dihydroxyphenylalanine; DA, dopamine; NE, norepinephrine; EPI, epinephrine; AADC, aromatic L-amino acid decarboxylase; PNMT: phenylethanolamine *N*-methyltransferase; VAMP: vesicle-associated membrane protein; Rab: member of RAS oncogene family; CADPS: Ca^{2+} -dependent secretion activator; CALM: calmodulin; SNAP: synaptosomal-associated protein. Adapted from Eisenhofer et al. (59).

Figure 4.

Receiver operating characteristic curves illustrating relationships between rates of true-positive test results (test sensitivity) and rates of false-positive test results (1-specificity) calculated at different upper reference limits for each of the tests. Curves for plasma free metanephrines (●) are shown in all panels A and B. Comparisons with plasma catecholamines (■), urinary catecholamines (△), and urinary VMA (▲) are shown in panel A and those with urinary fractionated metanephrines (○) and urinary total metanephrines (□) are shown in panel B. Note that at higher upper reference limits, rates of true-positive test results decrease (sensitivity decreases), whereas rates of false-positive test results increase (specificity increases). From Lenders et al. (87).

Figure 5.

Diagram illustrating the main pathways of catecholamine synthesis, release and metabolism in pheochromocytoma. Numbers in squares indicate sites of: (1) action of α - and β -adrenoceptor blockers or weight loss medications (phentermine (Adipex, Fastin, Zantryl), phendimetrazine (Bontril, Adipost, Plegine), methamphetamine (Desoxyn), and phenylethylamine (Fenphedra)) as sympathomimetic amines with a direct action on adrenoceptors; (2) Metyrosine (Demser) an inhibitor of TH; (3) inhibition of NE reuptake via NET (mainly on sympathetic nerve terminals) by the effect of tricyclic antidepressants (amitryptiline (*Elavil*, *Endep*), nortryptiline (*Aventyl*, *Pamelor*)), combined serotonin and NE reuptake inhibitors (duloxetine (*Cymbalta*), venlafaxine (*Effexor*)), sibutramine (*Meridia*), and cocaine; (4) where sympathomimetics (e.g. ephedrine, pseudoephedrine), and food containing tyramine (e.g., wine, cheese) release NE and EPI from storage vesicles; (5) MAO inhibitors (deprenyl (*Selegiline*)) blocking the conversion of NE to DHPG that results in accumulation and higher release of NE (mainly at sympathetic nerve terminals). TYR: tyrosine; DOPA: dihydroxyphenylalanine; DA: dopamine; DHPG: 3,4-dihydroxyphenylglycol; NE: norepinephrine; EPI: epinephrine; NMN: normetanephrine; MN: metanephrine; TH: tyrosine hydroxylase; PNMT: phenylethanolamine N-methyltransferase; MAO: monoamine oxidase; COMT: catechol-O-methyltransferase; NET: norepinephrine cell membrane transporter; AR: adrenoceptors. From Pacak (40).

Figure 6.

Mitochondria serves as home of many metabolic processes hence it is considered as the cell's "power plant". The transformation of a normal cell into a cancer cell (e.g. due to oxidative stress) results in dysfunction of many mitochondrial processes (so called "metabolic reprogramming") that favor a survival of any cancer cell. Dysfunctional mitochondria is responsible for many "cancer favoring" processes as sustained angiogenesis, inhibition of apoptosis, cancer cell invasion, limitless replication, etc. Note that in contrast to a normal cell that can well survive without any energy delivery (glucose and glutamine), a cancer cell dies quickly if deprived from these energy sources. Adapted from (180).

Figure 7.

Diagram illustrating consequences of succinate dehydrogenase (SDH) dysfunction (mutation) in pheochromocytoma. SDH mutation causes Krebs cycle dysfunction, enhanced glycolysis, stabilization of hypoxia inducible factor (HIF) and generation of reactive oxygen species (ROS). These main SDH-induced cellular alterations increase tumorigenesis, particularly in chromaffin cells (e.g. decreased apoptosis and increased angiogenesis, lipid, protein, and nucleotide synthesis, cell invasiveness and metastasis).

Table 1

Adrenergic receptors mediated responses of effector organs

Effector Organs	Adrenergic Impulses		Cholinergic Impulses
	Receptor Type*	Responses†	Responses†
Eye			
Radial muscle, iris	α_1	Contraction (mydriasis) ++	–
Sphincter muscle, iris		–	Contraction (miosis) +++
Ciliary muscle	β_2	Relaxation for far vision +	Contraction for near vision +++
Heart			
SA node	β_1, β_2	Increase in heart rate ++	Decrease in heart rate; vagal arrest + ++
Atria	β_1, β_2	Increase in contractility and conduction velocity ++	Decrease in contractility, shortened AP duration +++
AV node	β_1, β_2	Increase in automaticity and conduction velocity +++	Decrease in conduction velocity; AV block +++
His-Purkinje system	β_1, β_2	Increase in automaticity and conduction velocity +++	Little effect
Ventricles	β_1, β_2	Increase in contractility, conduction velocity, automaticity, and rate of idioventricular pacemakers +++	Slight decrease in contractility
Arterioles			
Coronary	$\alpha_1, \alpha_2; \beta_2$	Constriction +; dilations ++	Constriction +
Skin and mucosa	α_1, α_2	Constriction +++	Dilation
Skeletal muscle	$\alpha; \beta_2$	Constriction ++; dilations ++	Dilation +
Cerebral	α_1	Constriction (slight)	Dilation
Pulmonary	α_1, β_2	Constriction +; dilations,	Dilation
Abdominal viscera	α_1, β_2	Constriction +++; dilations +	–
Salivary glands	α_1, α_2	Constriction +++	Dilation ++
Renal	$\alpha_1, \alpha_2; \beta_1, \beta_2$	Constriction +++; dilations +	–
Veins (systemic)	$\alpha_1, \alpha_2; \beta_2$	Constriction ++; dilations + +	–
Lung			
Tracheal and bronchial muscle	β_2	Relaxation +	Contraction ++
Bronchial glands	α_1, β_2	Decreased secretion; increased secretion	Stimulation +++
Stomach			
Motility and tone	$\alpha_1, \alpha_2; \beta_2$	Decrease (usually) +	Increase ++
Sphincters	α_1	Contraction (usually) +	Relaxation (usually) +
Secretion		Inhibition (?)	Stimulation +++
Intestine			
Motility and tone	$\alpha_1, \alpha_2; \beta_1, \beta_2$	Decrease +	Increase +++
Sphincters	α_1	Contraction (usually) +	Relaxation (usually) +
Secretion	α_2	Inhibition	Stimulation ++
Gallbladder and ducts	β_2	Relaxation +	Contraction +
Kidney			

Effector Organs	Adrenergic Impulses		Cholinergic Impulses
	Receptor Type*	Responses†	Responses†
Renin secretion	α_1, β_1	Decrease +; increase ++	—
Urinary bladder			
Detrusor	β_2	Relaxation (usually) +	Contraction +++
Trigone and sphincter	α_1	Contraction ++	Relaxation ++
Uterus	α_1, β_2	Pregnant: contraction (α_1); relaxation (β_2); nonpregnant: relaxation (β_2)	Variable
Sex organs, male Skin	α_1	Ejaculation ++	Erection +++
Pilomotor muscles	α_1	Contraction ++	—
Sweat glands	α_1	Localized secretion +	Generalized secretion +++
Adrenal medulla		—	Secretion of epinephrine and norepinephrine (primarily nicotinic and secondarily muscarinic)
Skeletal muscle	β_2	Increased contractility; glycogenolysis; K^+ uptake	— —
Liver	α_1, β_2	Glycogenolysis and gluconeogenesis +++	
Pancreas			
Acini	α	Decreased secretion +	Secretion ++
Islets (β cells)	α_2	Decreased secretion +++	—
	β_2	Increased secretion +	—
Fat cells	$\alpha_2; \beta_1, (\beta_3)$	Lipolysis +++ (thermogenesis)	—

Abbreviations: SA, sinoatrial; AV, atrioventricular. Adapted from Pacak et al. (39).

Table 2

Discriminant Analysis for Classification of Patients According to Neurochemical Profile

Test or test combination	MEN 2 & NF1 vs. VHL & SDH	VHL versus vs. SDH
Percent Correctly Classified		
Plasma O-methylated metabolites		
NMN	47%	60%
MN	97%	50%
MTY	53%	78%
MN & NMN	99%	59%
MN & MTY	99%	79%
NMN & MTY	54%	78%
NMN & MN & MTY	100%	78%
Plasma catecholamines		
NE	55%	59%
EPI	81%	46%
DA	47%	61%
EPI & NE	84%	60%
EPI & DA	82%	59%
NE & DA	53%	65%
NE & EPI & DA	85%	70%
Urine Metanephrines		
NMN	50%	66%
MN	98%	57%
NMN & MN	98%	64%
Urine catecholamines		
NE	57%	60%
EPI	92%	62%
DA	61%	59%
EPI & NE	94%	61%
EPI & DA	93%	62%
NE & DA	67%	62%
NE & EPI & DA	94%	69%

Abbreviations: NMN, normetanephrine; MN, metanephrine; MTY, methoxytyramine; NE, norepinephrine; EPI, epinephrine; DA, dopamine. From Eisenhofer et al. (73)

Table 3

Emergencies due to catecholamine excess from pheochromocytoma

Clinical setting	Most prominent symptom(s)/sign(s)
Pheochromocytoma multisystem crisis	Multiple organ failure, temperature > 40°C, hypertension and/or hypotension
Cardiovascular	Collapse Hypertensive crisis - upon induction of anesthesia - medication induced or through other mechanisms Shock or severe hypotension Acute heart failure Myocardial infarction Arrhythmia Cardiomyopathy Myocarditis Dissecting aortic aneurysm Limb ischemia, digital necrosis or gangrene Deep vein thrombosis
Pulmonary	Acute pulmonary edema Adult respiratory distress syndrome
Abdominal	Abdominal bleeding Paralytic ileus Acute intestinal obstruction Severe enterocolitis and peritonitis Colon perforation Bowel ischemia Mesenteric vascular occlusion Acute pancreatitis Cholecystitis Megacolon Watery diarrhea syndrome with hypokalemia
Neurological	Hemiplegia General muscle weakness Generalized seizures
Renal	Acute renal failure Acute pyelonephritis Severe hematuria Renal artery stenosis by compression of tumor
Metabolic	Diabetic ketoacidosis Lactic acidosis

Adapted from Brouwers et al. (78).

Table 4

Potential oxidative and metabolic stress targets for the treatment of metastatic pheochromocytoma

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Inhibition of glycolysis:

Glucose uptake, glucose metabolism (via several enzymatic steps)
 Inhibition of lactate production (via lactate dehydrogenase type A)
 p53 pathway induction or restoration (also via TIGAR)
 Inhibition of PI3K/Akt pathway

Inhibition of glutaminolysis:

Glutamine uptake, glutamine metabolism (via several enzymatic steps)

Inhibition of the HIF-1 α stabilization and activity

Activation of prolyl hydroxylases
 Inhibition of HIF-1 α binding to DNA
 Reduction of the expression of HIF-1 α mRNA and protein (antisense)
 Inhibition of HIF-1 α activity
 Inhibition of PI3K/Akt pathway

Inhibition of reactive oxygen species (ROS) generation:

Antioxidants
 Activation of antioxidant genes
 Sestrins 1 and 4 induction
 p21 gene induction (including stabilization of Nrf2 transcription factor)

Mitochondrial proton pump modulators

Potassium channel enhancers - openers
 Na⁺/H⁺ pump inhibitors

Inhibition of fatty acid synthesis

Other mechanisms:

Activation of AMPK and TSC pathways, inhibition of mTORC pathway, inhibition of the HIF-2 α stabilization, NF κ B or cyclin D1