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New insights into nuclear imaging phenotypes of cluster 1 pheochromocytoma and paraganglioma

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Abstract

Pheochromocytomas and paragangliomas (PPGLs) belong to the family of neural crest cell-derived neoplasms. In up to 70% of cases, they are associated with germline and somatic mutations in 15 well-characterized PPGL driver or fusions genes. PPGLs can be grouped into three main clusters, where cluster 1 represents PPGLs characterized by a pseudohypoxic signature. Although cluster 1 tumors share several common features, they exhibit unique behaviors. Here, we present unique insights into imaging phenotypes of cluster 1 PPGLs based on glucose uptake, catecholamine metabolism and somatostatin receptor expression. Recent data suggests that succinate is a major player in the imaging phenotype of succinate dehydrogenase-deficient PPGLs. This review emphasizes the emerging stromal cell-succinate interaction and highlights new perspectives in PPGL theranostics.

Keywords

positron emission tomography; gallium radioisotopes; 68-Ga-DOTATATE; 6-(18F)fluoro-L-3,4-dihydroxyphenylalanine; somatostatin; pheochromocytoma; paraganglioma; radionuclide therapy; magnetic resonance; theranostics

Introduction

Pheochromocytomas and paragangliomas (PPGLs) belong to the family of neural crest cell derived neoplasms with an approximate annual incidence of 1 to 8 patients per million. They are widely distributed from the skull base to the urinary bladder and develop in close relationship with the sympathetic and parasympathetic divisions of the autonomic nervous

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Conflicts of interest

The authors have nothing to disclose.

system. Currently, almost 3/4 of these tumors are related to driver mutations in 15 well-characterized PPGL driver and fusion genes [1–3], about 27% of which are germline mutations and 46% somatic including fusions [3].

Recent data obtained from The Cancer Genome Atlas (TCGA) has currently provided us with the most sophisticated molecular taxonomy to date [3]:

1. **Pseudohypoxia** group (cluster 1) can be divided into at least two subgroups: tricarboxylic acid (TCA) cycle-related, containing germline mutations in succinate dehydrogenase subunits (*SDHx*) and fumarate hydratase (*FH*), a second enzyme in the tricarboxylic acid (TCA) cycle. The second subgroup: *VHL/EPAS1*-related, with somatic and germline mutations.
2. Wnt signaling group includes newly recognized somatic mutations in *CSDE1*, as well as somatic gene fusions affecting *MAML3*.
3. Kinase signaling group consists of germline or somatic mutations in *RET*, *NF1*, *TMEM127*, *MAX*, and *HRAS*.

Pseudohypoxic tumors exhibit a typical noradrenergic secretory profile (even when cluster 1 tumors are confined to the adrenal medulla) reflected by increases in plasma/urine norepinephrine or its metabolite normetanephrine. *SDHx* tumors are also characterized by the production of dopamine and its metabolite methoxytyramine, often secreted together with norepinephrine/normetanephrine [4].

SDHx genes comprise the *SDHA* [5], *SDHB* [6], *SDHC* [7], and *SDHD* [8] genes, which encode the four subunits of succinate dehydrogenase (collectively called SDH, also known as the mitochondrial complex II) [9], as well as *SDHAF2* (*SDHx*), assembly factor of the succinate dehydrogenase complex [10]. *SDHx* mutations also predispose patients to other tumors, particularly gastrointestinal stromal tumors (GISTs), renal cell carcinoma (RCC), and pituitary adenomas [11, 12].

Although *SDHx*-related PPGLs share common molecular and metabolic profiles, the clinical phenotype significantly varies across genotypes. Patients with *SDHB* mutations present more frequently with sympathetic (mostly extra-adrenal) PPGLs compared to patients with *SDHD* mutations who more commonly present with head and neck PGLs (HNPPGLs) with concomitant thoracoabdominal PGLs in only about 10% of cases. Patients with *SDHC* mutations typically harbor PGLs originating from the parasympathetic cervical or thoracic paraganglia [13]. *SDHA*-mutated PPGLs have been reported in a limited number of cases and are more commonly associated with the sympathetic system. The prevalence of malignancy is also variable, with a higher rate in *SDHB* (~30%) compared to *SDHA*, *SDHC*, and *SDHD* PPGLs (where occurrence of metastatic disease is rare, ~0–4%) [14]. Nevertheless, recent observations suggest that the rate of malignancy in *SDHA* PPGLs can be higher than previously expected and large scale studies are needed to establish the malignancy rate in these tumors (Pacak, unpublished observations) [15]. These distinguishing features illustrate that genotype alone cannot explain the entire clinical picture of *SDHx*-related PPGLs.

Von Hippel-Lindau syndrome is another pseudohypoxia-related disorder caused by germline mutations in the *VHL* gene [16]. This syndrome includes PPGLs that typically arise from the adrenal medulla (often bilateral) or much less frequently, extra-adrenal abdominal sympathetic paraganglia. In contrast, PPGLs linked to somatic *HIF2A* mutations occur sporadically. Exclusive to females, patients with somatic *HIF2A* mutations present with polycythemia, multiple PPGLs (sympathetic), and duodenal somatostatinomas [17]. These mutations are considered to occur during early embryogenesis in certain stem cells (mosaicism). Although *SDHx/VHL/HIF2A* belong to the same pseudohypoxia cluster 1, *SDHx*-related PPGLs exhibit different clinical and imaging phenotypes compared to *VHL/HIF2A*.

In recent years, other than the aforementioned *SDHx* mutations, the presence of mutations in other Tricarboxylic Acid (TCA) cycle (also called Krebs cycle) enzymes (Fumarate Hydratase (*FH*), Malate Dehydrogenase 2 (*MDH2*), Isocitrate Dehydrogenase 1 and 2 (*IDH1/2*)) have been identified in various cancers, signifying that these metabolic changes constitute an emerging metabolic hallmark of cancer [18]. Importantly, disruption of the TCA cycle is associated with accumulation of its intermediates that alter various cellular and extra-cellular functions. These supposed oncometabolites, particularly succinate and fumarate, influence a broad spectrum of pathways such as the hypoxic response, microenvironment and immune system functions, invasiveness, metastasis and epigenetic reprogramming [19–27]. Thus, the major outcome of elevated succinate and fumarate levels is HIF stabilization and development of a hypermethylation profile (via inhibition of 2-oxoglutarate-dependent histone and DNA demethylases). Both of these are tightly linked to tumorigenesis of chromaffin cells that give rise to PPGLs. DNA hypermethylation also silences key genes involved in neuroendocrine differentiation (such as *Phenylethanolamine N-Methyltransferase (PNMT)*), leading to a noradrenergic secretory profile [19]. Most recently, succinate-stromal cell interaction has been described to explain the specific imaging phenotype seen in *SDHx*-related PPGLs [28].

Here, we provide an overview of current and emerging imaging phenotypes in cluster 1 PPGLs with a specific focus on the role of succinate on the uptake of ^{18}F -2-fluoro-2-deoxy-D-glucose (^{18}F -FDG) (see Glossary) by stromal cells, which is translated to the well-proven observation of positive ^{18}F -FDG PET in *SDHx*-related PPGLs [28].

A. Pseudohypoxia ^{18}F -FDG imaging phenotype

SDHx-related PPGLs exhibit a highly elevated ^{18}F -FDG uptake compared to their sporadic or other hereditary counterparts. The link between metabolic reprogramming associated with high oncometabolite levels and ^{18}F -FDG PET imaging phenotype, in these and other tumors, has been extensively debated and several hypotheses, including this new one, are now presented.

The main, and so far the most accepted hypothesis, relies on the stabilization of HIF proteins despite a normal oxygen supply (referred to as the pseudohypoxia hypothesis) resulting in induction of glucose uptake and consumption due to increased glycolysis despite normal oxygen supply (Warburg effect) [29–32]. HIF-1 α and HIF-2 α form heterodimers with the β

subunit. HIF proteins are primarily regulated by stabilization of the alpha subunits depending on oxygen availability. Under normoxic conditions, HIF- α subunits are rapidly degraded by the proteasome. The activity of HIF- α subunits is regulated by prolyl hydroxylation of their oxygen-dependent degradation domain. Prolyl hydroxylation targets the proteins for proteasomal degradation by promoting the interaction of HIF with the VHL protein, a component of the E3 multiprotein ubiquitin-ligase. These hydroxylation events are catalyzed by prolyl hydroxylase domain proteins (PHDs). When oxygen concentration decreases, PHDs become inactive and the HIF proteins are consequently stabilized. The accumulation of succinate observed in *SDHx*-mutated PPGL inhibits PHDs. Thus, PHD inhibition by succinate or *VHL/HIF2A* mutations creates a pseudohypoxic state resulting in upregulation of HIF target genes [33] (Box 1). Although HIF-1 α and HIF-2 α share some redundant functions (glucose transport, pH homeostasis, angiogenesis), they also exhibit unique functions such as the regulation of the expression of the glycolytic enzymes and PDK1 for HIF-1 α and cellular iron metabolism for HIF-2 α [34]. The importance of the HIF signaling pathway in angiogenesis and tumorigenesis [34, 35] is also demonstrated by the association between PPGLs and mutations in the key proteins of the oxygen sensing pathway (i.e., *VHL*, *EPAS1/HIF2A*, *FH* [36], *PHD1/EGLN2* [37], *PHD2/EGLN1* [38] and *MDH2* [39]). Transcriptomic studies have demonstrated that although both HIF-1 α and HIF-2 α target genes are overexpressed in the *SDHx/VHL* subclusters, HIF-1 α target genes are predominantly activated in *VHL*-mutated tumors compared to *SDHx* tumors, where HIF-2 α is considered to be paramount [40]. Increased ^{18}F -FDG uptake in cluster 1 PPGLs was, until now, explained by an increase in the expression of glucose transporters and/or glycolytic enzymes, especially hexokinases, which catalyze the first committed step of glucose metabolism [31].

Clear cell RCC (representing another example of a pseudohypoxic tumor) overexpresses both HIF-1 α and HIF-2 α and their ratio varies based on tumor type, genotype, and other yet uncovered mechanisms [41]. Most of these tumors carry somatic *VHL* inactivation (in >90% of cases); however, they often fail to concentrate ^{18}F -FDG. Our group also failed to identify any difference in ^{18}F -FDG uptake between the TCA cycle-related *IDH1*-mutated and wild-type gliomas [42].

B. Emerging stromal cell-succinate- ^{18}F -FDG imaging phenotype

Fluxomic studies that determine the rates of metabolic reactions show that pharmacological inhibition of SDH or *SDH* knockdown alone does not impair cellular respiration because it is still sustained in redox equivalents by glutaminolysis and succinate secretion flux [43]. Beyond succinate accumulation, it has been shown that *SDH* knockout cells rely on increased pyruvate carboxylase (PC)-dependent aspartate production and reductive glutamine metabolism [44–46]. Glutaminolysis and pyruvate carboxylation are two anaplerotic pathways (i.e. chemical reactions that form intermediates of a metabolic pathway).

Some *in vitro* experiments have also shown counter-intuitive results. *SDHB*-mutated neuroblastoma cell lines were found to have a paradoxical decrease in glucose uptake compared to wild-type cells despite an increased growth rate and invasiveness [47]. These

effects were even more pronounced in the presence of human fibroblasts in co-culture experiments. Primary human fibroblasts exhibit an increased glucose uptake when they are co-cultured with wild-type cells, and an even greater uptake when co-cultured with *SDHB*-silenced neuroblastoma cell lines. Activation of HIF target genes promotes tumorigenesis and tumor aggressiveness, but the relationship with ^{18}F -FDG phenotype remains unclear. Beyond the Warburg effect which may occur in some cases, how does SDH deficiency connect with ^{18}F -FDG PET/CT?

Since *in vitro* findings suggest a possible metabolic cooperation between stroma and cancer cells [48], the identification of a potential metabolic mediator between these two compartments is becoming increasingly important. *SDHx*-related PPGLs dramatically accumulate succinate as a result of a TCA blockade that is detectable by *ex vivo* [49–51] and *in vivo* proton MR spectroscopy [52, 53].

Therefore, it was hypothesized that succinate could be the connecting hub between *SDHx* mutation status and ^{18}F -FDG uptake. This effect would be mediated by a succinate efflux for *SDHx* mutated cells. Analyses of compartmentalized levels of TCA cycle metabolites have revealed that yeast with *sdh* mutations may aberrantly efflux succinate from the mitochondria to the cytosol [54]. Other studies have even reported that succinate could be excreted into the medium during cultivation of yeast *sdh* mutants [55, 56]. It has been speculated that this retrograde pathway may prevent the potential detrimental effects of succinate excess on non-mitochondrial processes [57]. Succinate can migrate through the mitochondrial and plasma membrane via different transport systems such as a succinate-fumarate transporter in the inner mitochondrial membrane, porins in the outer mitochondrial membrane and a sodium-independent anion exchanger in the plasma membrane. Although these transporters have not been characterized in humans, succinate efflux is likely happening and, therefore, succinate is likely to act as an extracellular ligand. This efflux of succinate is also presumed in patients with *SDHx*-related PPGLs presenting with a higher level of plasma succinate-to-fumarate ratio compared to apparently sporadic and neurofibromatosis type 1 patients [58]. It was recently shown that an intratumoral injection of succinate in xenograft tumors induces an increase of ^{18}F -FDG uptake, unlike with a PBS and fumarate injection [28]. Interestingly, this effect was not observed when cancer cells were co-cultured with succinate, suggesting an effect of succinate on stromal cells. Finally, this demonstrated that endothelial cells were sensitive to succinate and that intramuscular injection of succinate also induced ^{18}F -FDG uptake, highlighting the effect of succinate on connective tissues. It is of note that succinate could have hormone-like actions in blood cells, fat, liver, heart, retina and kidney through activation of receptor GPR91 [59]. Although succinate promotes the development of new blood vessels following ischemia, we demonstrated that succinate-induced ^{18}F -FDG uptake was not due to increased blood flow or increased capillary permeability [60]. These new findings suggest that succinate stimulates ^{18}F -FDG uptake through endothelial cells, further supporting our previous discovery, now very well accepted by oncologists, that *SDHx*-PPGLs show high positivity on ^{18}F -FDG-PET scans [61] and this approach has been now recommended by the US Endocrine Society [62] (Figure 1).

C. Catecholamine metabolism-imaging phenotype

¹²³I-Metaiodobenzylguanidine (¹²³I-MIBG), ¹¹C-Hydroxyephedrine (¹¹C-HED) and ¹⁸F-Fluorodopamine (¹⁸F-FDA) illustrate the uptake and storage properties of catecholamines of PPGLs. L-3,4-dihydroxyphenylalanine (L-DOPA) (is an intermediate in the catecholamine synthesis pathway, where it can be decarboxylated to dopamine by amino acid decarboxylase (AADC) and then concentrated in intracellular vesicles. Unlike ¹²³I-MIBG/¹¹C-HED/¹⁸F-FDA which enter the cells via norepinephrine transporter, ¹⁸F-FDOPA is taken up via neutral amino acid transporter system L (Figure 1). System L transports large hydrophobic amino acids in a sodium-independent manner. System L is heteromeric and is composed of a heavy glycoprotein chain (CD98hc, encoded by *SLC3A2*) and one of two catalytic L chains, LAT1 or LAT2, encoded by *SLC7A5* and *SLC7A8* genes, respectively. PPGLs express LAT1 and or LAT2 and are therefore targetable with ¹⁸F-FDOPA. ¹⁸F-FDOPA PET/CT was found to have high sensitivity and specificity in patients with PPGLs [68]. Tumor uptake is significantly correlated with levels of metanephrines (unpublished personal observations, David Taïeb). The presence of an *SDHx* mutation markedly influences the catecholaminergic phenotype [69]. ¹⁸F-FDOPA PET/CT sensitivity is decreased in *SDHx*-related PPGLs. ¹⁸F-FDOPA PET/CT can miss metastases [69] or primaries, especially those arising from the sympathetic paraganglia [70]. This intra-patient tumor heterogeneity in *SDHx*-mutated patients is currently unexplained. It is remarkable to note that this phenomenon exhibits an all-or-none pattern, meaning that these tumors will either uptake, or not uptake at all, the ¹⁸F-FDOPA tracer. We have recently shown that the decreased expression in CD98hc/LAT1 proteins explains ¹⁸F-FDOPA PET negativity in a small number of cases [71]. It should be noted that LAT1 and LAT2 act as exchangers [72], for example, ¹⁸F-FDOPA enters into the cells via an obligatory efflux of certain amino acids. Since it was shown that *SDHx*-mutated cells display changes in the intracellular pool of aminoacids, we therefore hypothesized that these changes could reduce activity of transporters. Absence of ¹⁸F-FDOPA uptake could be related to the combination TCA defect and secretory activity of tumor cells. However, this remains to be established in further studies. In contrast, ¹⁸F-FDOPA PET is highly sensitive in *VHL*, *HIF2A*, and *FH* PPGLs [73].

D. Somatostatin receptors-imaging phenotype

PPGLs overexpress somatostatin receptor type 2 and, therefore, can be targeted by radiolabeled somatostatin analogs (SSAs) for imaging. In contrast to gastroenteropancreatic neuroendocrine tumors (GEP-NETs), PPGLs only slightly express subtype 5. In recent years, several ⁶⁸Ga-labeled-SSAs (DOTATOC: Tyr3-octreotide, DOTATATE: Tyr3-octreotate, and DOTANOC: Nal3-octreotide) have been developed for PET imaging of somatostatin receptors and have shown excellent sensitivity in the evaluation of HNPGLs and metastatic PPGLs [74, 75]. Surprisingly, metastatic PPGLs associated with *SDHB* mutations, which are more aggressive than their sporadic counterparts, exhibit intense uptake of ⁶⁸Ga-labeled-SSAs [76–78]. These findings were somewhat counterintuitive in comparison to GEP-NETs that usually exhibit a flip-flop phenomenon with high ¹⁸F-FDG/low labeled-SSA uptake pattern in aggressive forms [79]. However, this has considerably simplified the imaging approach in a PPGL patient. *EPAS1* mutations remain

an exception among susceptibility genes since they lead to PPGLs that concentrate less ^{68}Ga -labeled-SSA in contrast to *SDHx*-related PPGLs [80]. This phenotype is currently largely unexplained.

E. Transfer of knowledge on phenotypic imaging of cluster 1 PPGLs into clinical practice

Based on the currently available PET imaging techniques, we propose the following approach to investigate a patient with cluster 1 PPGL (Table 1):

1. For diagnosis of PPGLs in doubtful situations, the specificity provided by ^{18}F -FDOPA PET/CT or ^{68}Ga -DOTA-SSAs is superior to other radiopharmaceuticals.
2. For detecting multifocality or metastases, both *SDHx* and non-*SDHx* PPGLs, are better visualized by ^{68}Ga -DOTA-SSA than ^{18}F -FDOPA PET/CT or even ^{18}F -FDG PET/CT.
3. For patients with a high-risk of developing tumors limited to the adrenal glands with potential multifocality (*i.e.*, *RET*, *MAX*, *NF1*), ^{18}F -FDOPA PET/CT should be used as the first-line approach due to the higher tumor-to-background uptake ratio.
4. In PPGLs associated with direct activation of the hypoxia signaling pathway (*EPAS1*, *PHD1*, and *PHD2*), ^{18}F -FDOPA PET/CT is the imaging modality of choice.

Future Perspectives

Nuclear imaging-based disease phenotyping provides a better understanding of PPGLs with 4 major imaging phenotypes: a pseudohypoxia ^{18}F -FDG imaging phenotype common to all cluster 1 tumors; a stromal cell-succinate ^{18}F -FDG imaging phenotype linked to the presence of SDH deficiency; a catecholamine metabolism-imaging phenotype dependent on tumor content of catecholamines and the secretion of their metabolites, thereby providing information on their functional differentiation; and somatostatin receptors-imaging phenotype that further characterizes PPGLs and enables selection of patients for peptide receptor radionuclide therapy using somatostatin analogs labeled with therapeutic radioisotopes (Figure 2).

The future of precision medicine in PPGLs also depends on the identification of new PPGL cell specific targets (Box 2). However, their utilization for imaging or therapy will require a more expanded understanding of the disease at a molecular level and the intimate relationship between genotype-metabotype and imaging phenotypes. Recent data indicate that intermediate (onco)metabolites may act as mediators between tumor cells and stroma. A better understanding of these “metabolic synapses” between tumor and other (stromal) cells will be a great challenge over the next few years and molecular imaging is ideally placed to play a central role in this regard. Interestingly, a recent study has shown that succinate has pro-angiogenic functions (chemotactic motility, tube-like structure formation and proliferation) and also upregulates vascular endothelial growth factor expression [67, 81].

This concept also supports a notion that succinate may contribute to the aggressive behavior potential of *SDHx* tumors through the new concept outlined here and in our previous publication, affecting various stromal cells and ultimately promoting metastasis. Furthermore, succinate-endothelial cell interaction could be used to monitor PPGL response or failure during or after treatment using various angiogenesis inhibitors where a failure of such treatment would not be associated with lower FDG uptake as seen in successful angiogenic therapy. Thus, integrated ^{18}F -FDG **positron emission tomography (PET)**/MR proton spectroscopy (MRS) could therefore become an important radiomics approach in the evaluation of antiangiogenic agents but also the evaluation of specific therapies against *SDHx* deficient PPGL, enabling assessment of *in vivo* succinate concentration by MRS and endothelial metabolic response to succinate by ^{18}F -FDG PET. Finally, in the near future **theranostics** uniquely linked to the role of the immune system in a cancer cell will undoubtedly bring new twists on how we defeat various tumors, including PPGLs.

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Glossary

Nuclear imaging

an imaging technique that enables noninvasive visualization of a wide variety of physiological and pathological processes at the metabolic and molecular level. To do so, a biologically active molecule (vector) labeled with a diagnostic radionuclide (probe or isotope) is used in very small quantities (radiotracer) in order to be concentrated in the studied biological processes/tissues without disturbing them. For specific molecular targeting, the vector should be suited to specific PPGL cell characteristics.

Positron emission tomography (PET)

a nuclear imaging technology based on the detection of pairs of photons emitted indirectly by a positron-emitting radionuclides (e.g., ^{18}F , ^{68}Ga) via an electron-positron annihilation reaction.

Theranostics

an approach that encapsulates the integration of diagnostic and *therapeutic* functions within the *same* platform of pharmaceuticals (theranostic pair). In the theranostic paradigm, it is an implicit assumption that results derived from an imaging study using a compound labeled with a diagnostic radionuclide can precisely determine whether an individual patient is likely to benefit from a specific treatment using the same or a related compound labeled with a therapeutic radionuclide.

^{18}F -2-fluoro-2-deoxy-D-glucose (^{18}F -FDG)

a radioactive glucose analog which is taken up by cells via glucose membrane transporters and phosphorylated by hexokinase into ^{18}F -FDG-6P. ^{18}F -FDG-6P does not follow further enzymatic pathways and therefore, it accumulates in cells including tumors in proportion to the rate of carbohydrate metabolism.

Pseudohypoxia

a condition characterized by upregulation of hypoxia-inducible factors (HIFs)-targeted genes in cells, under normoxia, due to inappropriately stabilized HIF proteins (HIFs escape to proteasome degradation and it is translocated into nucleus). Stabilization of HIF- α subunits is related to deregulation of the ubiquitin/proteasome system (*SDHx*, von Hippel-Lindau-

VHL, or Endothelial PAS Domain Protein 1-EPAS-1 (also called *Hypoxia-Inducible Factor 2 Alpha-HIF2A*) mutations.

⁶⁸Ga-labeled somatostatin analogs

radiopharmaceuticals that enable *in vivo* assessment by PET imaging of somatostatin receptors expression on a cell including tumors. ⁶⁸Ga is produced from a ⁶⁸Ge/⁶⁸Ga generator and extemporaneous coupled to DOTA-somatostatin analogs for clinical detection of tumors. The current importance of ⁶⁸Ga-labeled somatostatin analogs as theranostic radiopharmaceuticals lies in their dual application as imaging agents as well as therapeutic radioisotopes (such as ⁹⁰Y and ¹⁷⁷Lu).

Box 1. Succinate: beyond the tricarboxylic acid cycle

Succinate (succinic acid in blood pH), for many decades, has been considered only as an intermediate metabolite of the tricarboxylic acid (TCA) cycle. During aerobic respiration, succinate is oxidized to fumarate, donating reducing equivalents. The reaction is catalyzed by succinate dehydrogenase (SDH), an enzyme complex located in the inner mitochondrial membrane that participates in both the TCA cycle and electron transport chain. Hans Adolf Krebs team noticed that some intermediates, including succinate, could accumulate in the interstitial space during liver ischemia [63]. During ischemia, succinate can be produced by reduction of fumarate (a purine nucleotide cycle metabolite) via the reverse action of SDH. Succinate is then secondarily secreted from the cells into the blood stream [64]. Many studies have shown that succinate has several functions beyond participating in the TCA cycle, of which some are mediated via a G protein-coupled succinate receptor (GPR91), an orphan molecule that belongs to the G protein-coupled receptor (GPCR) family [65]. Through GPR91, succinate may have hormone-like actions in blood cells as well as in fat, liver, heart, retina and kidney [59]. For instance, in response to retinal ischemia, succinate plays an important role in the development of new blood vessels via GPR91 and subsequent modulation of VEGF release by retinal ganglion neurons [60].

In 2000, Baysal et al. described the first PGL syndrome (PGL1) related to a SDH deficiency, due to a mutation in the *SDHD* [8]. Later, several familial clusters of PPGLs related to deleterious mutations in any of *SDHx* were described and defined as PGL syndromes PGL1 through PGL4. From a biochemical standpoint, they invariably result in decreased SDH activity and highly elevated concentrations of succinate, due to TCA blockade. Succinate is capable of acting as an intracellular and extracellular messenger. Succinate leads to a set of distinct features that enhance tumor growth and survival via HIF proteins stabilization with increased expression of HIF-target genes (angiogenesis), hypermethylation profile that is viewed as a contributing factor to both tumor aggressiveness (epithelial to mesenchymal transition) and loss of chromaffin-specific patterns of gene expression. Succinate also exacerbates inflammation [66]. Recently, succinate was also shown to promote angiogenesis (chemotactic motility, tube-like structure formation and proliferation) through a HIF-independent pathway, by activating ERK1/2 signaling via GPR91 in endothelial cells [67].

Box 2. Pheochromocytoma and paraganglioma: tumors born to be imaged

The major advantage of nuclear imaging over other imaging modalities is to provide unique opportunities for better characterization of these tumors at the molecular level. Given the overexpression of a wide variety of specific targets in PPGLs, it seems that these tumors ideally suited to be imaged by specific radiopharmaceuticals. Indeed, this opportunity has more recently been augmented by a number of excellent radiopharmaceuticals, which target different functional and molecular pathways that often reflect the diverse genetic landscape of PPGLs (e.g., catecholamine synthesis, somatostatin receptors and amino acid transporter expression), mirroring *ex vivo* histological classification but on a whole-body, *in vivo*, scale. The ability to detect PPGLs by molecular imaging goes hand-in-hand with its ability to characterize the molecular metabolomics and epigenetic features of these tumors. The diversity of available radiopharmaceuticals is an advantage for the current management of patients, regardless of the approach being used. It is expected that information provided by novel imaging studies will offer promising new results that will provide a better understanding of the disease and determine optimal treatment options. Beyond the expected clinical benefits of personalized medicine, theranostics could also have a significant positive economic effect. The development of new radiopharmaceuticals and PET/Magnetic Resonance-MR (including MR-based metabolomics) for tumor characterization (imaging biomarkers) and the development of therapeutic nuclear medicine will offer new avenues in the personalized approach to PPGL patients.

Trends

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors arising from neural crest derivatives (paraganglia) that can be distributed throughout the body.

In recent years, genetics has profoundly helped gain a more comprehensive pathogenic picture to PPGLs. Based on data from The Cancer Genome Atlas (TCGA), PPGLs can be stratified into three main clinically relevant clusters, Cluster 1 PPGLs (pseudohypoxic) being associated with specific molecular, biochemical, and imaging features.

This comprehensive approach is now translated into a precision medicine approach in a patient's management including diagnosis, therapies, follow-ups, and the surveillance/counseling of family members.

Nuclear medicine has emerged at the forefront of precision medicine. Nuclear imaging-based phenotyping of PPGLs enables identification of different metabolic-imaging phenotypes that provide better understanding of PPGL in a given patient; these can be integrated into the patient's personalized management, including the use of therapeutic radionuclides and possibly their survival outcome.

Outstanding questions

What is the mechanism by which succinate increases ^{18}F -FDG uptake ?

Are the effects of pseudohypoxia distinguishable from the effects of succinate on stroma cells in ^{18}F -FDG imaging phenotypes ?

Can ^{18}F -FDG uptake by cluster 1 PPGLs be viewed as a marker of endothelial metabolic activity and therefore be used to monitor a response to anti-angiogenic therapy ?

Is the manipulation of succinate's effects on endothelial cells associated with antiangiogenic effects in cluster 1 PPGLs ?

What causes phenotypic imaging differences between tumors that coexist in a single patient? Could radiolabeled succinate and other TCA intermediates potentially be used for functional imaging of cluster 1 PPGLs ?

Could PPGL specific radiopharmaceuticals provide a synergistic therapeutic approach together with immunotherapy and antiangiogenics ?

Do the various levels of succinate in cluster 1 PPGLs affect their responses to peptide receptor radionuclide therapy ?

Figure 1. Schematic display of succinate targets in *SDHx*-related PPGLs and mechanisms of uptake of PET radiopharmaceuticals

Glucose is taken up by normal cells via GLUT transporters (GLUTs). Glucose is metabolized via glycolysis to generate pyruvate that undergoes mitochondrial oxidation by the tricarboxylic acid (TCA). Cellular energy status (i.e., ATP levels) is maintained primarily through the electron transport chain and oxidative phosphorylation. *SDHx*-related mutated cells are characterized by decreased SDH activity with TCA blockade and accumulation of highly elevated concentrations of succinate. Succinate is capable of acting as an intracellular and extracellular messenger. Succinate leads to a set of distinct features via HIF proteins stabilization, despite normal oxygen supply (pseudohypoxia) with increased expression of HIF-target genes (angiogenesis, proliferation, metabolic adaptation), DNA hypermethylation (dedifferentiation, epithelial to mesenchymal transition). Succinate was also shown to promote angiogenesis and activate ^{18}F -FDG uptake by endothelial cells. ^{18}F -FDG imaging phenotype of *SDHx*-related PPGLs can therefore be related to HIF-dependent (pseudohypoxia) and independent (stromal cell-succinate interaction) mechanisms. L-DOPA enters into the neutral amino acid transporter System L (LAT1 or LAT2), which acts as an exchanger and is decarboxylated into dopamine by amino acid decarboxylase (AADC); then it is concentrated in catecholamine storage vesicles where it can be further metabolized into norepinephrine (NE) and epinephrine (E). Catecholamines are released by exocytosis. ^{18}F -FDOPA imaging phenotype is dependent on expression and/or activity of catecholamine synthesizing enzymes (differentiation pattern). *SDHx*-related PPGLs are

characterized by decreased ^{18}F -FDOPA uptake, due to dedifferentiation and possibly a decrease in intracellular content of aminoacids that are involved in the ^{18}F -FDOPA influx. ^{68}Ga -labeled-SSA uptake is mainly dependent on somatostatin receptors expression. Somatostatin receptor agonists trigger internalization of the ligand receptor-complex, whereas somatostatin receptor antagonists did not but could be better candidates to target tumors. *SDHx*-related PPGLs exhibit ^{68}Ga -labeled-SSA uptake. PHD : prolyl hydroxylase domain proteins, VHL : Von Hippel-Lindau protein, VEGF : Vascular endothelial growth factor, TH : tyrosine hydroxylase.

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Figure 2. PET-based imaging phenotyping in a patient with metastatic pheochromocytoma
23-year-old *SDHB* patient who was first diagnosed with right adrenal pheochromocytoma at the age of 10 presents to us with metastases (hot spots) to the lung (long arrow), liver (short arrow), retroperitoneum, and skeleton. PET images using 4 different radiopharmaceuticals are presented: ^{68}Ga -DOTATATE (A), ^{18}F -FDG (B), ^{18}F -FDOPA (C), and ^{18}F -FDA (D). ^{68}Ga -DOTATATE identifies more metastatic lesions than ^{18}F -FDG (B) whereas ^{18}F -FDOPA (C) shows a doubtful uptake foci in the right lower lung nodule, marked by a long arrow, and ^{18}F -FDA (D) is essentially negative. This imaging feature is typical of *SDHB*-related metastatic PHEO.

Table 1

Current proposed PET radiopharmaceuticals for PPGL imaging according to genetic background

	Location	Other related tumor conditions	First-choice radiopharmaceutical	Second-choice radiopharmaceutical
<i>SDHB</i>	Adrenal/extradr enal	GISTs, RCCs and Pituitary adenomas	⁶⁸ Ga-DOTA-SSAs	¹⁸ F-FDG
<i>SDHD</i>	Adrenal/extradr enal	GISTs, RCCs and pituitary adenomas	⁶⁸ Ga-DOTA-SSAs	¹⁸ F-FDG
<i>SDHC</i>	Adrenal/extradr enal	GISTs	⁶⁸ Ga-DOTA-SSAs	¹⁸ F-FDG
<i>FH</i>	Adrenal/extradr enal	Uterine leiomyomas and RCCs	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs
<i>VHL</i>	Adrenal/extradr enal	RCCs, CNS hemangioblastomas, Pancreatic and testicular tumors	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs
<i>EPAS1/HIF 2A</i>	Adrenal/extradr enal	Somatostatinomas	¹⁸ F-FDOPA	¹⁸ F-FDG
<i>MEN2</i>	Adrenal	MTC, parathyroid adenomas or hyperplasia	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs
<i>NF1</i>	Adrenal	Neurofibromas, peripheral nerve sheath tumors and gliomas	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs
<i>TMEM127</i>	Adrenal	RCCs	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs
<i>MAX</i>	Adrenal/extradr enal	Renal oncocytomas	¹⁸ F-FDOPA	⁶⁸ Ga-DOTA-SSAs