Chapter

Metastatic Paragangliomas and Pheochromocytomas: An Epigenetic View

María-Dolores Chiara, Lucía Celada, Andrés San José Martinez, Tamara Cubiella, Enol Álvarez-González and Nuria Valdés

Abstract

Paragangliomas and pheochromocytoma (PPGLs) are hereditary tumors in about 40% of cases. Mutations in the genes encoding for components of the mitochondrial succinate dehydrogenase protein complex (*SDHB*, *SDHD*, *SDHC*) are among the most prevalent. Most PPGLs have a benign behavior, but patients with germline *SDHB* mutations may develop metastatic PPGLs in up to 30% of cases. This suggest that the SDH substrate, succinate, is key for the activation of the metastatic cascade. The last decade has witnessed significant advances in our understanding of how succinate may have oncogenic properties. It is now widely accepted that succinate is an oncometabolite that modifies the epigenetic landscape of SDH-deficient tumors via modulating the activities of DNA and histone modification enzymes. In this chapter, we summarize recent discoveries linking SDH-deficiency and metastasis in SDH-deficient PPGLs via inhibition of DNA methylcytosine dioxygenases, histone demethylases and modified expression of non-coding RNAs. We also highlight promising therapeutic avenues that may be used to counteract epigenetic deregulations.

Keywords: paraganglioma, pheochromocytoma, metastasis, epigenetic, DNA methylation, histone methylation, succinate

1. Introduction

Paragangliomas and pheochromocytomas (PPGLs) are rare neuroendocrine tumors that originate in the diffuse paraganglionic tissue and the adrenal gland, respectively. Approximately 40% of these tumors are hereditary and related to germline mutations in *SDHB, SDHC, SDHA*, *SDHD* and *SDHAF2* (collectively called *SDHx*), as well as *RET, VHL, NF1, TMEM127, MAX, FH, KIF1B* and *EGLN1* among others [1]. Mutations in genes encoding different subunits of the succinate dehydrogenase (SDH) complex are the most prevalent in hereditary PPGLs being present in about 50% of cases. Among these genes, *SDHB*, *SDHD* and *SDHC* are the most frequently affected. Somatic mutations affecting *SDHx* genes can be also detected in non-hereditary PPGLs [2]. The strong association of *SDHx* mutations and PPGLs reveals that the activity of this mitochondrial complex plays an

essential, and likely unique, role in the neuroendocrine tissues conforming human paraganglia such that its deregulation cause development of neoplasia in these tissues that can, eventually, become metastatic.

One of the peculiarities of PPGLs is that they are generally slow growing, indolent tumors that are not life-threatening. However, 10–30% (according to different studies) of the PPGLs metastasize and once metastasis occurs, treatment options are rather limited and patients have poor prognosis, often with less than 50% surviving at 5 years [3]. Surgery can improve the prognosis but standard chemotherapeutic regimen with cyclophosphamide, vincristine, and dacarbazine, or radionuclide therapy with 131 Iodine-radiolabelled metaiodobenzylguanidine result in only partial responses. Thus, there is still a long road to reach therapeutic improvements. Further challenges for clinicians come from the fact that, in half of the cases, metastases are not present during the initial treatment of the patient but emerge over a period of undetermined time, which may even exceed 10 years after diagnosis of the primary tumor. For this reason, these patients receive long-term, post-treatment surveillance. However, the duration as well as the interval of the follow-up screening is poorly defined. Following these reasonings, the WHO 2017 Classification of Tumors of Endocrine Organs stated that PPGLs should be considered as tumors of undetermined biologic potential and should not be termed benign but should be classified as metastatic or not metastatic [4]. Given that all PPGLs are recognized as exhibiting malignant potential to some extent, the risk for malignant behavior must be determined to be able to pinpoint cases at risk of future metastases directly in the early post-operative period, a knowledge that would have a significant clinical impact.

Despite overwhelming advances in understanding the molecular mechanisms of PPGL development made in the last decade, the factors governing the emergence of metastasis are still very poorly understood. Considerable efforts have been made in identifying histopathological features suggestive of metastatic behavior using pre-defined algorithms. The Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) and the Grading System for Adrenal Pheochromocytoma and Paraganglioma (GAPP), rely on different histopathologic features or on a combination of histopathologic, immunohistochemical (Ki-67 index) and biochemical (catecholamine production) parameters, respectively, as tools to distinguish PPGLs with potential for aggressive behavior [5]. However, these algorithms lack accuracy and have a high degree of inter-observer variability thus complicating their clinical roll-out. Hence, the guiding of therapeutic decision-making by using predictive biomarkers in PPGL patients require in-depth knowledge of the biology of this neoplasia.

2. Epigenetic and SDH-deficiency: a connection with metastatic potential

The metastatic cascade involves a succession of cell phenotypic alterations that spans from the acquisition of local invasive activity, the intravasation of cancer cells into blood and lymphatic vessels, their subsequent extravasation in the parenchyma of distant tissues and finally their growth forming macroscopic tumors. How a primary PPGL-tumor cell becomes metastatic and what are the molecular events involved in this process remain to be known. With the emergence of genomic profiling technologies, single gene/protein or multi-gene "signature"-based assays have been introduced to measure specific molecular pathway deregulations in cancer which could be used as clinically useful biomarkers. In PPGLs' patients, it is well established that the presence of inactivating germline mutations in the

SDHB gene is the most important molecular predictor of malignancy. More than 40% of patients with metastatic PPGLs (especially extra-adrenal tumors) carry germline *SDHB* mutations [6, 7]. Although mutations in other PPGL-predisposing genes, such as *FH*, *SDHC*, *SDHD*, *SDHA,* and *TMEM127* have been found in some patients with metastatic PPGLs, these mutations account for only <5% of cases. The mitochondrial 2-oxoglutarate/malate carrier *SLC25A11* gene has been proposed as a novel gene that can confer a predisposition to metastatic PPGLs but the number of patients harboring *SLC25A11*-germline mutations was rather limited to definitely assigned it a role in metastasis development [8]. Thus, *SDHB* gene germline mutation remains as the most reliable risk factor for metastasis. Nonetheless, metastases are developed in only 30% of the *SDHB*-mutation carriers and it is not known what are the mechanisms that either tip the balance towards the metastatic process or prevent it in these patients. Recent studies have pointed to several cancer-related genetic deregulations in metastatic PPGLs, especially prevalent in *SDHB*-related tumors. These include activation of telomerase and over-expression of genes involved in epithelial to mesenchymal transition [9–11]. However, these molecular alterations have been found in limited number of metastatic PPGLs and it is not known what their role is as triggers of the metastatic process. Aside *SDHB*-related metastatic PPGLs, the specific genetic traits involved in the development of the remaining 60% of metastatic PPGLs are not known. Somatic mutations in *ATRX* and *SETD2* genes, and fusions of *MAML3* gene have been identified in metastatic PPGLs [12, 13].

One of the most relevant hints on the molecular mechanisms involved in metastasis came from the The Cancer Genome Atlas (TCGA) Program. These studies revealed that metastatic *SDHx*-mutated PPGLs do not accumulate more gene mutations at the somatic level than no-metastatic PPGLs [13]. It is now becoming increasingly evident that epigenetic changes play a key role in providing properties to the primary cancer cell that have a major contribution to the metastatic process. Relevant studies revealed that PPGLs, developed in patients with mutations in *SDHx* genes, harbor a DNA hypermethylation phenotype which is not present in PPGLs developed in patients with other genetic backgrounds [14]. Although these variations are commonly found in benign and metastatic *SDHB*-mutated PPGLs, qualitative and/or quantitative deviations could cooperate to set the trigger for metastasis development.

Epigenetics is defined as heritable changes in gene expression that do not involve a change in DNA sequence. Epigenetic changes occur in many types of cancer cells and include DNA methylation, histone modification, and small RNAs. Aberrant hypermethylation can lead to silencing of tumor-suppressor genes, histone modifications control the accessibility of the chromatin and transcriptional activities inside a cell, and microRNAs (miRNAs) can negatively control their target gene expression post-transcriptionally. Herein, we provide a perspective on the recent advances and challenges in our understanding of how epigenetic deregulations may underlie the progression of SDH-deficient PPGLs towards a metastatic disease and highlight promising therapeutic avenues that may be used to counteract those epigenetic deregulations.

3. Succinate: an oncometabolite driving epigenetic deregulation in SDH-deficient PPGLs

The SDH complex links the tricarboxylic acid cycle (TCA) and the mitochondria respiratory chain by the coupling of succinate oxidation to fumarate to the reduction of ubiquinone to ubiquinol at the mitochondrial complex II (**Figure 1**). The

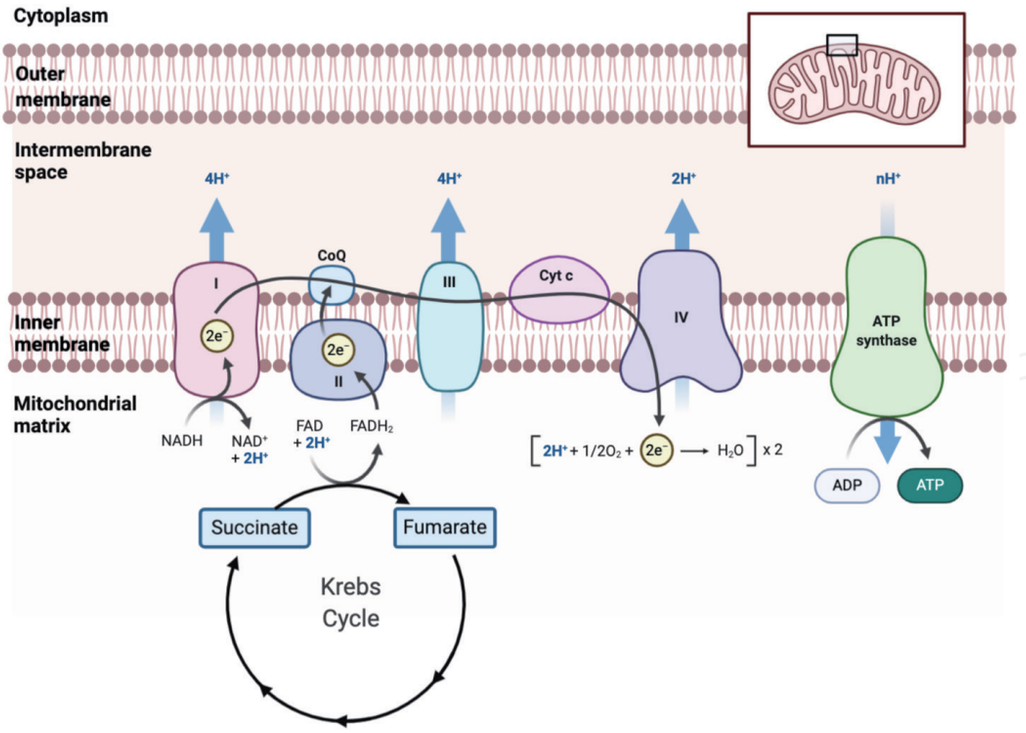

Figure 1.
Schematic representation of the SDH-mediated connection between the Krebs cycle and the mitochondrial respiratory chain. The succinate dehydrogenase complex is part of both, the Krebs cycle at the mitochondria matrix and the mitochondria respiratory chain in the inner mitochondrial membrane. It is composed of four subunits (SDHA, SDHB, SDHC and SDHD) that couples the succinate oxidation to fumarate to the reduction of ubiquinone (coenzyme Q: CoQ) to ubiquinol via FAD at the mitochondrial complex II. The mitochondria respiratory chain consists of four membrane-bound, multimeric protein complexes (complexes I, II, III, and IV) that catalyzes the oxidation of reducing equivalents, mainly nicotinamide adenine dinucleotide (NADH), using the terminal electron acceptor oxygen. This electron transfer is linked to the ATP synthase, which generates ATP.

fumarate/succinate ratio and the redox state of the ubiquinone pool act as signal transducers known to modulate the regulatory programs that control cell fate. Loss of SDH activity leads to dramatic elevation of its natural substrate, succinate. The succinate generated in the mitochondrial matrix is exported to the cytosol where it can inhibit 2-oxoglutarate (2OG)-dependent dioxygenases such as ten-eleven translocation (TET) DNA cytosine-oxidizing enzymes and prolyl hydroxylases (PHD) [15].

PHD enzymes catalyze the prolyl-hydroxylation of the hypoxia-inducible factors HIF1α and HIF2α which transcriptionally regulates HIFα-responsive genes and conform the major hub involved in oxygen-sensing (**Figure 2**). These genes serve to adapt cells to oxygen deficiencies and their over-activation under pathologic conditions may also have pro-tumorigenic activity. HIFα proteins are degraded under physiological conditions by a mechanism requiring active PHD enzymes. PHD-catalyzed prolyl-hydroxylation of HIFα proteins is required by their recognition by VHL, subsequent ubiquitination and proteasomal degradation. Low oxygen levels and succinate repress PHD activities thus leading to the stabilization and functional activation of HIFα proteins. This oxygen-sensing pathway has long been considered a driver mechanism of metastasis in tumors with SDH-deficiencies [16]. However, although HIF1α protein and HIF1α-responsive genes are over-expressed in PPGLs carrying *SDHx* mutations, this signature is much weaker than that of PPGLs carrying *VHL*-loss-of-function mutations which rarely metastasize [17, 18]. Moreover, nuclear HIF2α does accumulate in all paragangliomas of the head and neck which very scarcely develop metastasis. These observations argue against nuclear HIFα

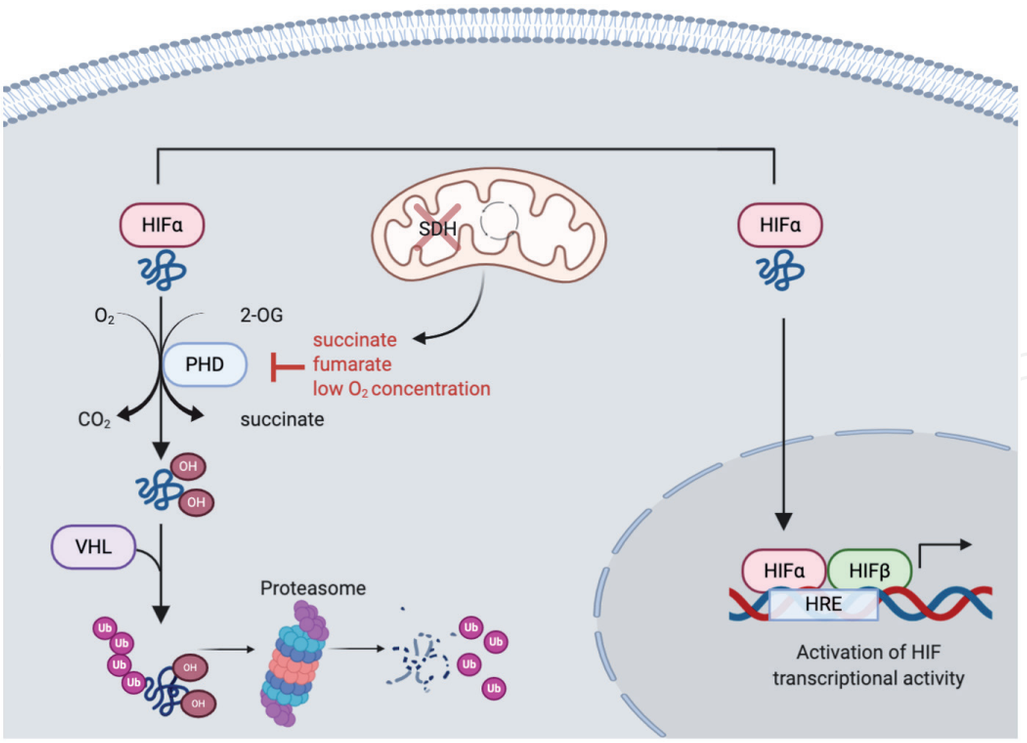

Figure 2.
Oxygen and oncometabolite dependent regulation of HIFα. Under physiological conditions, prolyl hidroxylases (PHD) hydroxylate two proline residues in HIFα subunits thus allowing their recognition by the von Hipple-Lindau protein (VHL). VHL is a component of a ubiquitination protein complex that ubiquitinate (Ub) prolyl-hydroxylated HIFα for degradation by the proteasome. PHDs activity rely on oxygen (O_2) and oxoglutarate (2-OG). When oxygen concentration diminishes below physiological levels the activity of PHDs is inhibited leading to the dissociation of VHL from HIFα which results in HIFα stabilization that is transported to the nucleus, binds to HIFβ and activates transcription of target genes by binding to hypoxia-responsive elements (HRE) in their promoter regions. Succinate, as well as fumarate, structurally mimics 2-OG and inhibits PHDs (product inhibition) when present at elevated concentrations, as observed in tumor cells carrying inactivating mutations-driven disfunction of SDH or fumarate hydratase.

proteins as the triggers of malignant transformation of *SDHx*-mutant PPGLs. Further research is required to demonstrate whether any, both or none of the HIFα proteins are required for malignant transformation of PPGLs.

In addition to PHDs, succinate, which can accumulate to millimolar levels in *SDHx*-mutant PPGLs, is a potent inhibitor of TET enzymes and the Jumonji domain-containing histone demethylases [19]. TET enzymes hydroxylate DNA-methylcytosines into 5-hydroxymethylcytosine leading to DNA demethylation. Increased DNA methylation in or near promoter regions, and subsequent decreased gene expression, has been associated with oncogenesis in a number of tumor types including PPGLs carrying *SDHx*-mutations [14]. A role for TET enzymes in this phenotype has been recently demonstrated [20].

In addition to DNA epigenetic alterations, metastasis in PPGLs patients has also been shown to be associated with other epigenetic traits such as aberrant expression of long non-coding RNA (lncRNA) [21] and microRNAs (miRNAs) [22, 23] although these deregulations are not specific of SDH-deficient metastatic PPGLs.

3.1 Succinate-induced DNA hypermethylation

Site-specific DNA hypermethylation in regions of DNA with a high density of cytosine-guanine (CpG) dinucleotides in promoters represent a common feature of the cancer-associated epigenetic landscape. These CpG hypermethylations are linked with repressive chromatin modifications and silencing of tumor suppressor

genes. We discuss here the current understanding of the epigenetic basis of metastasis in *SDHB*-related PPGLs uncovered by our recent studies.

To identify epigenetic alterations relevant for metastasis, we recently performed a comprehensive analysis of DNA methylation in metastatic PPGLs with and without *SDHB* mutations. This analysis revealed that over 1000 genes harbored promoter hypermethylation in the metastatic tumors but not in the not metastatic ones thus suggesting that those gene alterations have a role in the pathogenesis of the metastatic disease linked to *SDHB* mutations [24]. About 15% of these alterations had been also identified in *sdhb−/−* mouse chromaffin cells and in 41% of *SDHx*-mutated PPGLs analyzed by Letouzé et al. [14]. Although these authors did not make distinctions whether the PPGLs were or not metastatic, they did find that hypermethylation was stronger in *SDHB*-PPGLs. Therefore, it is likely that gene

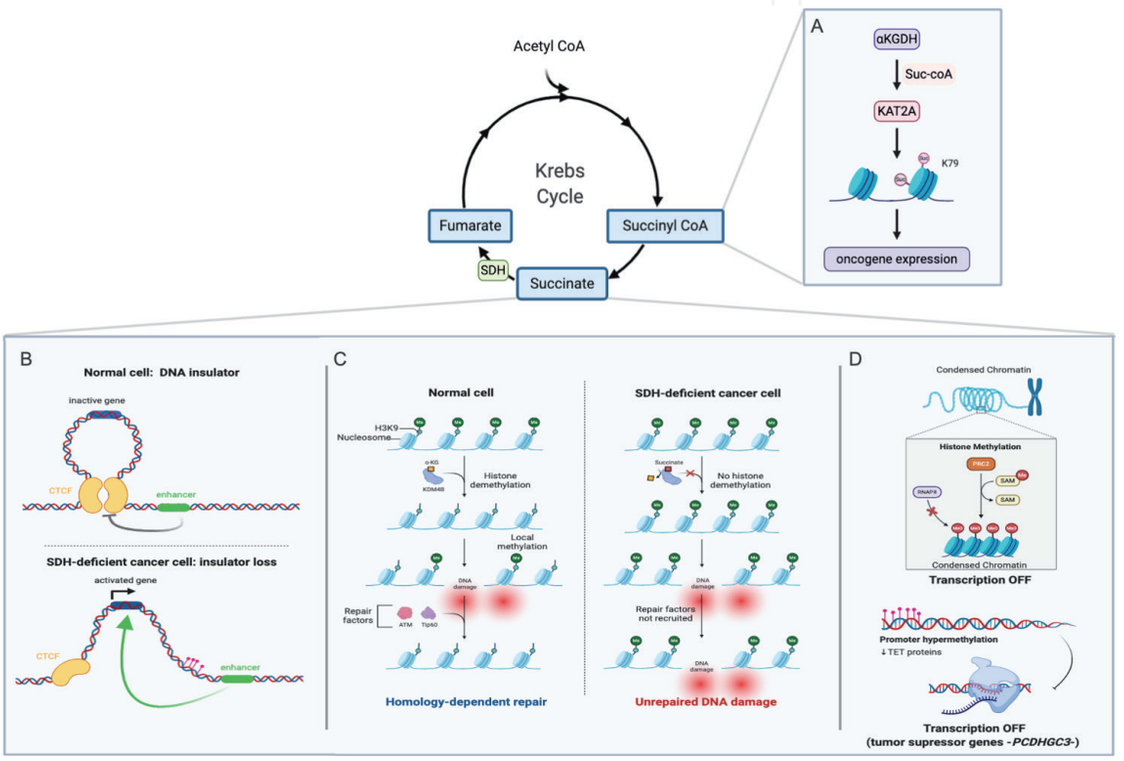

Figure 3.
Outline of the epigenetic changes induced by abnormal succinate accumulation due to SDHx-mutations. Mutations of the SDHx genes in PPGLs cause blockage of SDH activity and subsequent abnormal succinate and succinyl-CoA accumulation. Increased levels of succinate induce inhibition of 2-oxoglutarate-dependent dioxygenases such as TET enzymes and the Jumonji domain-containing histone demethylases leading to activation or repression of gene transcription. TET enzymes hydroxylate DNA-methylcytosines into 5-hydroxymethylcytosine leading to DNA demethylation. Increased DNA methylation due to impaired TET enzyme functions in or near promoter regions induces decreased gene transcription (D) that, when affects tumor suppressor genes, such as PCDHGC3, may trigger different aspects of the metastatic programs. Gene expression can also be inhibited by succinate-induced inhibition of Jumonji domain-containing histone demethylases that remove the methyl group on lysine in histone tails. Histone methylation occurs by the transfer of methyl groups from the methyl donor S-adenosylmethionine (SAM) to amino acids of histone proteins. This protein modification can either increase or decrease transcription of genes, depending on which amino acids are methylated, and how many methyl groups are attached. Succinate inhibition of demethylation of trimethylated-H3K27 by the Polycomb complex (PRC2) induce gene silencing and chromatin condensation (D). (C) Succinate also represses homology-dependent DNA repair by inhibiting the H3K9 demethylase, leading to global elevation of trimethylated H3K9 chromatin marks at loci surrounding DNA breaks. This masks a local H3K9 trimethylation signal that is essential for the proper execution of homology-dependent DNA repair. (A, B) Apart from repression of gene expression, abnormal succinate accumulation may induce gene transcription. This occurs when DNA methylation affects CTCF insulators which prevents CTCF binding to CTCF binding sites, CTCF dimerization and the assembly of long-range chromatin looping. This provokes promiscuous enhancer-promoter interactions and the subsequent induction of the affected genes (B). (A) Increased succinyl-CoA levels induce succinylation of histones associated with enhanced in vitro transcription. The figure shows the enzymatic succinylation of histone via the KAT2A histone succinyltransferase which associates with α-ketoglutarate dehydrogenase (α-KGDH).

mutations in *SDHB* induce epigenetic programs that may be involved in tumors initiation and others involved in metastasis development.

Gene set enrichment analysis revealed that the hypermethylated promoters in metastatic *SDHB*-mutated PPGLs were associated with developmental genes that are preferential targets of the polycomb repressive complex 2, PRC2. PRC2 catalyzes the mono-methylation, di-methylation and tri-methylation of histone H3 at lysine 27 required for PRC2-mediated gene silencing and for maintaining cellular identity during differentiation and development [25]. Specifically, PRC2 occupies a special set of developmental genes in embryonic stem cells that must be repressed to maintain pluripotency and that are poised for activation during cell differentiation. In cancer, aberrant promoter hypermethylation, or PRC-mediated repression, can inhibit differentiation programs, such that cancer cells are arrested at a proliferative state [26] (see **Figure 3**). In agreement with these observations, increasingly, metabolites, such as succinate, are recognized as important modulators of the regulatory programs that control cell fate [27]. Thus, it is tempting to speculate that the succinate 'oncometabolite' plays an essential role in the epigenetic reprogramming of chromaffin cells such that, when reaching high enough levels, induces the transit of mature differentiated cells towards a less differentiated state that allow them to proliferate and generate a tumor mass. This could provide an explanation for tumorigenesis in SDH-deficient tumors. However, it cannot explain why some SDH-deficient PPGLs acquire metastatic fitness, but others do not. The identification of an epigenetic signature specific for metastatic SDH-deficient PPGLs, but not present in SDH-PPGLs that do not develop metastasis, provides some clues. Our recent study revealed that, in addition to the epigenetic changes in developmentally regulated genes, high level hypermethylation of genes involved in homophilic cell-to-cell adhesion was present in metastatic but not in non-metastatic PPGLs *SDHB*-mutated PPGLs. Loss of cell–cell adhesion is a hallmark of metastatic cells required for the transformation of immobile cells into motile cells providing them the ability to invade local tissues leading to metastasis at distant organs. Among these hypermethylated genes, we identified *CNTN2*, *SDK1*, *TENM1*, *TENM4* encoding neuronal cell adhesion molecules involved in the establishment of connections in the nervous system. More strikingly, the cell–cell adhesion set of hypermethylated genes included a 1 Mb-long chromosomal region that hold clustered protocadherin genes [designated as *PCDHA*, *PCDHB* and *PCDHG* (collectively, *PCDHs*)] encompassing 50 different genes at the chromosomal locus 5q31.3 [24] (**Figure 4**). One of the *PCDH* genes, *PCDHGC3*, has been further analyzed and found to be of clinical relevance in metastatic *SDHB*-mutated PPGLs.

3.2 Long-range hypermethylation of clustered protocadherin genes in metastatic *SDHB*-mutated PPGLs

PCDH genes, organized into three closely linked gene clusters (*PCDHA*, *PCDHB* and *PCDHG*), span nearly 1 million base pairs [28] (**Figure 4**). The *PCDHA* and *PCDHG* clusters are organized into variable and constant exons. The generation of full-length *PCDHA* and *PCDHG* messenger RNA requires RNA splicing of each variable exon to three constant exons. Each of the variable exon promoters are randomly activated in individual neurons to generate individual cell-specific patterns of *PCDH* gene expression. In contrast, *PCDHB* mRNA consists of only the variable exon. These genes are involved in the regulation of neural development and engage in homophilic/heterophilic trans-interactions as multimers acting as cell-surface molecular barcodes [29–33]. Their unique genomic organization makes them sensitive to long range epigenetic silencing (LRES). Several recent studies have revealed that epigenetic silencing of clustered *PCDHs* is

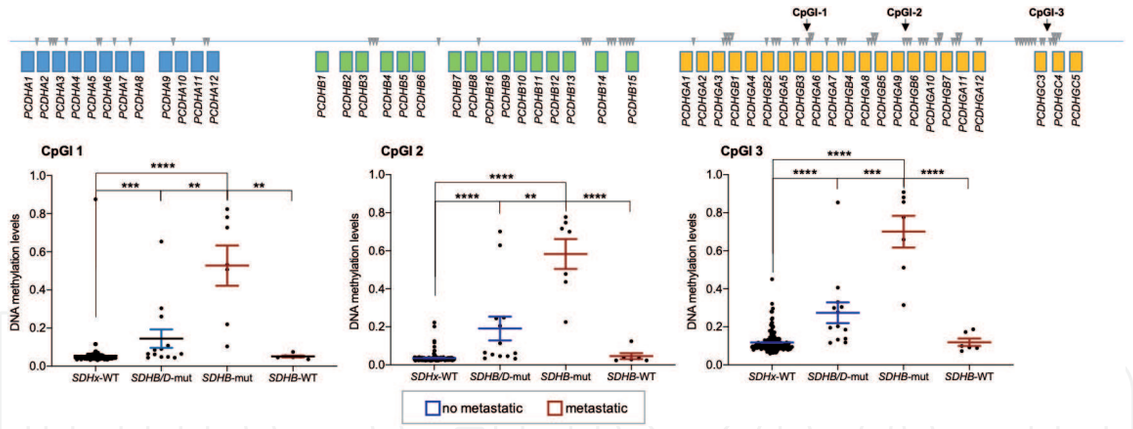

Figure 4.
High level long-range hypermethylation of the clustered PCDH genes in metastatic SDHx-mutated PPGLs. Schematic representation of the genomic organization of the clustered PCDHA, PCDHB and PCDHG genes. For PCDHA and PCDHG genes, only the first exons (blue and orange rectangles, respectively) are represented. For PCDHB genes, rectangles represent the whole gene. Inverted gray triangles point to CpG hypermethylation sites detected in the SDHx-mutated PPGLs included in the TCGA database. Graphics represent DNA methylation levels of the indicated CpG islands (CpGI) according to their genotype. Data from patients without or with metastasis are represented in blue and red, respectively. SDHx-WT: PPGLs lacking mutations in any of the SDHx genes (include PPGLs with and without mutations in other PPGL-susceptibility genes); SDHB/D-Mut: Metastatic PPGLs from patients with germline mutations in SDHB *or SDHD genes; SDHB-Mut: PPGLs from patients with germline mutations in SDHB genes; SDHB-WT: Metastatic PPGLs lacking mutations in SDHB genes.* ** P < *0.01;* *** P < *0.001;* **** P < *0.0001.*

present in various human malignant tumors, such as Wilms tumor, neuroblastoma, breast, prostate, colon cancer, gastric and biliary tract cancers, and astrocytoma suggesting that this process plays roles in regulating cancer development and/or progression [34–37]. By using one of the largest cohorts of epigenetically studied *SDHB*-mutated PPGLs, we have recently found that the epigenetic silencing of one of the clustered *PCDH* genes, *PCDHGC3*, is putatively involved in the metastatic behavior of these tumors [24]. Methylation of *PCDHGC3* promoter were found to be null in normal paraganglia, null or low in most *SDHB*-mutated PPGLs that do not metastasize, high in *SDHB*-mutated metastatic PPGLs, and much higher in the metastatic tissues derived from these tumors. Similar findings have been reported in colorectal cancer, showing that *PCDHGC3* is methylated and silenced during the adenoma-to-carcinoma transition [37]. These data suggest that this epigenetic trait is progressively amplified during the transformation of the tumor cells from benign state to the invasive and metastatic states, as suggested for other oncogenes and tumor suppressor genes [38].

We also found that, not only *PCDHGC3*, but the other clustered *PCDH* genes are highly methylated in metastatic SDHx-mutated PPGLs. Indeed, the *in-silico* analysis of DNA methylation data reported by TCGA confirmed the hypermethylation of the clustered *PCDH* genes (**Figure 4**) in *SDHx*-mutated PPGLs and allowed further analysis of this phenomena. As in our report, methylation of different CpG islands were detected in the three clustered *PCDHs*, being more highly enriched in the *PCDHG* cluster. **Figure 4** shows analysis of three different CpG regions in that cluster revealing that, similarly to our findings in *PCDHGC3* promoter region, methylation levels were higher in *SDHx*-mutated PPGLs than in PPGLs that did not harbor *SDHx* mutations. More importantly, among the *SDHB*-mutated PPGLs, those having a metastatic behavior had a significantly higher levels of methylation than tumors that had not developed metastasis at the last follow-up date. Analysis of the RNAseq data confirmed the epigenetic silencing of, not only *PCDHGC3* [24], but also *PCDHGC4* gene (**Figure 5**). The *PCDHGC4* mRNA levels were found significantly decreased in *SDHx*-mutated PPGLs as compared with tumors with other

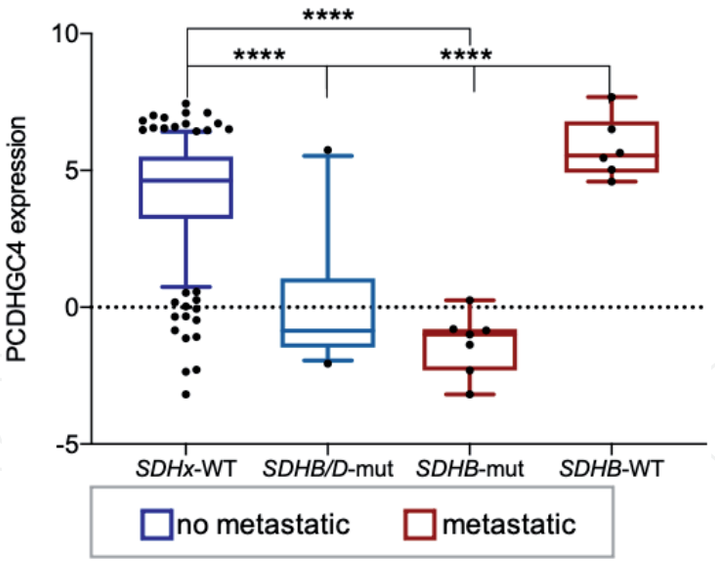

Figure 5.
PCDHGC4 *gene silencing in SDHB-mutated PPGLs that developed metastasis. PCDHGC4 mRNA levels in metastatic (red) and not-metastatic (blue) PPGLs included in the TCGA database are represented according to their genotype. SDHx-WT: PPGLs lacking mutations in any of the SDHx genes (include PPGLs with and without mutations in other PPGL-susceptibility genes); SDHB/D-Mut: PPGLs from patients with germline mutations in SDHB or SDHD genes; SDHB-Mut: Metastatic PPGLs from patients with germline mutations in SDHB genes; SDHB-WT: Metastatic PPGLs lacking mutations in SDHB genes.* **** P < *0.0001.*

genotypes. More importantly, downregulation was significantly more dramatic in metastatic than in benign *SDHx*-mutated tumors. Thus, the corrupted epigenetic changes in this chromosomal region seems to amplify the positive selection of the most metastatic cells and the evolutionary capacity of cancer to spread out of the tissue of origin. Interestingly, only the PCDHGC4 isoform, among the clustered PCDH proteins, have been shown to be strictly required for postnatal viability and survival of many neuronal subsets [39].

Consistent with previous findings in colon cancer cell lines [37], we found in that decreased *PCDHGC3* gene expression in two different cancer cell lines resulted in significant increases in cell proliferation, cell migration, and collective cell invasion. Silencing of *PCDHGC3* gene also resulted in increased tumor growth in studies of xenograft tumor models *in vivo*. Consistent with this, the current published data showed that PCDHs regulate pathways for cell proliferation and death. In tumor tissues derived from PPGLs, loss of *PCDH* expression is an indicator of poor prognosis, as revealed by our data and the *in silico* analysis of published data. Importantly, in *SDHB*-mutated metastatic PPGLs with high levels of *PCDHGC3* methylation, diagnosis of primary tumor and metastatic disease was synchronous in most cases, but some patients had a metastasis-free time ranging from 1 to 19 years. Thus, it is possible that epigenetic alterations of *PCDHGC3* during tumor initiation do not automatically lead to the manifestation of full metastatic potential. Rather, metastatic potential likely evolves through quantitative amplification, ultimately providing the cell with metastatic fitness. Thus, *PCDHGC3* acts as a tumor suppressor gene in PPGLs, could be an efficient biomarker of malignancy, and could represent a novel target for personalized medicine.

Targeting any of the protocadherin genes is challenging given that they are highly expressed in nervous system where exert relevant functions for the establishment and maintenance of specific neuronal connections. It is imperative, thus, to unravel the signaling pathways downstream *PCDHGC3* to identify potential therapeutic targets activated in the absence of *PCDHGC3* expression. Current published data have shown that PCDHs are tightly linked to several major signaling pathways, including the Wnt/β-catenin and receptor tyrosine kinase signaling

pathways [40–44]. In renal cancer cell lines, we have found that *PCDHGC3* loss of expression associates with increased mTOR activity. Several reports have shown activation of the mTOR pathway in PPGLs [45]. In addition, inhibition of this pathway exerts potent antitumor activity in a rat model of pheochromocytoma [46]. The epigenetic silencing of *PCDHGC3* could, thus, serve as a biomarker for the selection of patients appropriate for therapeutic options targeting the mTOR pathway.

3.3 Succinate-induced histone methylation

Gene expression can also be altered by changes in chromatin structure via chemical modification of amino acids on histone tails. Accumulation of high levels of succinate in SDH-deficient PPGLs inhibits JmjC domain-containing histone demethylases (KDMs) [19, 47, 48]. These KDMs remove the methyl group on lysine in histone tails, which can either activate or repress transcription depending on the specifically modified lysine residues. Generally, H3K4, H3K36 and H3K79 methylations are considered to mark active transcription, whereas H3K9, H3K27 and H4K20 methylations are thought to be associated with silenced chromatin states [49].

Succinate increases methylation of H3K27 and H3K79 [19]. Trimethylation of H3K27 is a hallmark of repressed transcription. It is tightly associated with inactive gene promoters and also the gene promoters that were found hypermethylated in *SDHB*-mutated metastatic PPGLs. Instead, H3K79 methylation is linked to active transcription and may influence transcription elongation and genomic stability [50] (**Figure 4**).

Succinate induces inhibition of the activities of KDM4A which remove methylation on histone 3 lysine 9 (H3K9) [51, 52]. H3K9 methylation is the mark of heterochromatin, which is the condensed, transcriptionally inactive state of chromatin. Importantly, Sulkowski et al. have recently shown that increased succinate levels, induced by SDH silencing, can also repress homology-dependent DNA repair (HDR) by directly inhibiting the H3K9 demethylase KDM4B, leading to global elevation of trimethylated H3K9 chromatin marks at loci surrounding DNA breaks. This masks a local H3K9 trimethylation signal that is essential for the proper execution of HDR [51] (**Figure 4**). This finding underscores the notion that decreased DNA repair acts as a key oncogenic mechanism in SDH-deficient PPGLs, similarly to the underlying mechanisms of the familial breast and ovarian cancer predisposition syndromes linked to the *BRCA1* and *BRCA2* genes.

3.4 Succinate-induced loss of insulators

DNA hypermethylation outside of gene promoters may also have significant impacts on PPGL pathophysiology, especially when hypermethylation occurs at the CCCTC-binding factor (CTCF) insulators. Insulators are DNA regulatory elements that block the interaction between gene enhancers and gene promoters. They block the spreading of enhancers action and thus insulate, or shield, gene promoters from unwanted regulation [53, 54]. CTCF dimerization, when it is bound to different DNA sequences, mediates long-range chromatin looping allowing the insulation of promoters from enhancer sequences (**Figure 4**). Many proto-oncogenes are isolated in such domains and thus protected from promiscuous enhancer interactions. The CTCF insulator is methylation-sensitive and may be displaced by DNA methylation. DNA hypermethylation at CTCF insulators is traduced in promiscuous enhancer-promoter interactions with the subsequent induction of the affected genes [53, 55].

Recent studies of SDH-deficient gastrointestinal stromal tumors (GISTs) have uncovered the frequent hypermethylation of CTCF insulators where DNA methylation replaces CTCF binding [55, 56]. This ubiquitous insulator losses leads SDH-deficient cells to acquire promiscuous enhancer-promoter interactions and an altered genome topology promoting expression of genes such as *FGF4* or *KIT* involved in the oncogenic programs activated in GIST. This discovery raises the interesting possibility that SDH-deficiency in PPGLs may drive oncogenic programs, in the absence of DNA mutations, by epigenetic modifications that alter genome topology and the enhancer/promoter functions.

3.5 Succinate-induced protein succinylation

SDH inactivation induces accumulation of the immediate upstream metabolite, succinyl-CoA. Succinyl-CoA is the substrate used for the succinylation of proteins, in which succinyl group is transferred to a lysine residue of a protein. It is a recently identified common and widespread posttranslational modification that directly couples TCA cycle metabolism, via succinyl-CoA, to alterations in the structures and activities of proteins involved in diverse cellular processes [57].

Lysine succinylation can occur by a non-enzymatic chemical reaction. This suggests that the abundance of succinyl-CoA would be one of the main governing factors of protein succinylation. A recent study have demonstrated that knockdown of *SDHB* leads to global lysine hyper-succinylation in multiple cellular compartments, especially mitochondria, coupled with increased succinyl-CoA levels [58]. Succinate-induced hypersuccinylation results in apoptosis resistance suggesting a relevant role in tumorigenesis and metastasis development. Succinylation can also occur at the nuclei. In this regard, Wang et al. have demonstrated that the lysine acetyltransferase 2A (KAT2A) may also act as a histone succinyltransferase by forming a complex with α-ketoglutarate dehydrogenase (α-KGDH) that catalyzes the conversion of α-ketoglutarate (α-KG) to succinyl-CoA in the promoter regions of genes [59] (**Figure 4**). Indeed, more than one-third of nucleosomes, including histone and non-histone chromatin components, have been shown to be lysine succinylated in the absence of functional SDH activity suggesting that SDH loss has significant effects on chromatin structure and function and subsequent gene expression [60]. These succinyl marks in chromatin coincide with H3K4me3-chromatin marks, but not with H3K27me3-chromatin marks, suggesting that succinylation of chromatin at active gene promoters is functionally meaningful. Histone succinylation induces widespread gene expression changes that promote tumor growth [61, 62]. However, how histone and nonhistone protein succinylation affects tumorigenesis remains largely unexplored and deserve in-depth characterization to unravel their putative involvement in metastasis development in patients with *SDHB*-mutations and to develop drug therapies and targeted agents.

4. microRNA and lncRNA

RNA-based mechanisms of epigenetic regulation are less well understood than mechanisms involved on DNA methylation and histones but have also profound roles in gene regulation, development and tumorigenesis. Several recent studies have analyzed the pattern of expression of non-coding RNAs, including microRNAs (miRNAs) and long-non-coding RNAs (lncRNAs), in metastatic PPGLs.

Mature miRNAs (~22 nucleotides long) base-pair with target mRNAs to inhibit translation or direct mRNA degradation. Several studies have shown over-expression of miR-183 in metastatic compared with non-metastatic PPGLs, irrespective of

the genotype of the tumor [23, 63]. Higher levels of miR-483-5p have been also in metastatic tumors compared with benign tumors [23, 64]. Given the rarity of PPGLs, in general, and of metastatic PPGLs with *SDHB* mutations, in particular, the putative involvement of SDH-deficiency mediated miRNA deregulation in metastasis development is yet unknown.

miR-210 is one of the best characterized miRNAs downstream HIF1α activation and a candidate tumor-driver of metabolic reprogramming in cancer [65]. Some studies have proposed that up-regulation of miR-210 is a hallmark of the *VHL*/*SDHx*-mutated PPGLs [66] whereas others have ascribed it a role exclusively in *VHL*-mutated tumors [18]. One of the targets of miR-210 is the gene that codifies the iron–sulfur cluster assembly enzyme (*ISCU*) required for the assembly of maturation of Fe-S clusters, critical bioinorganic prosthetic groups essential for electron transport and multiple metabolic processes [67]. The miR-210-*ISCU* signaling pathway, a hallmark of the HIF activation in cancer, is activated in *SDHx* and *VHL*-mutated PPGLs [18]. However, the role of miR-210 in metastasis predisposition of *SDHB*-mutated PPGLs is not known. A recent report showed that the serum levels of miR-210 are decreased in metastatic PPGLs [68] although these data were grounded in a very limited number of samples and has not been confirmed in publicly available databases. For example, *in silico* analysis of the TCGA database confirms previous reports showing that miR-210 is highly over-expressed in *VHL*-mutated PPGLs and moderately up-regulated in *SDHB*-PPGLs although this was independent on whether the tumor had or not metastatic behavior (**Figure 6**). Similarly, *ISCU* mRNA levels more dramatically decreased in *VHL*-mutated than in *SDHx*-mutated PPGLs. Among *SDHB*-mutated PPGLs, the differences of *ISCU* levels were not significant enough to assign it a role as biomarker of metastasis development. miR-210 was not found over-expressed, neither was *ISCU* under-expressed, in tumors carrying somatic mutations of the gene encoding the HIF2α subunit (*EPAS1*) of the HIF transcription factor thus confirming previous reports showing that this miRNA is a substrate of HIF1α but not HIF2α [69], at least, in the context of paraganglionar tissues. Thus, the available data suggest that miR-210 should not be used as a biomarker of metastatic *SDHB*-mutated PPGLs.

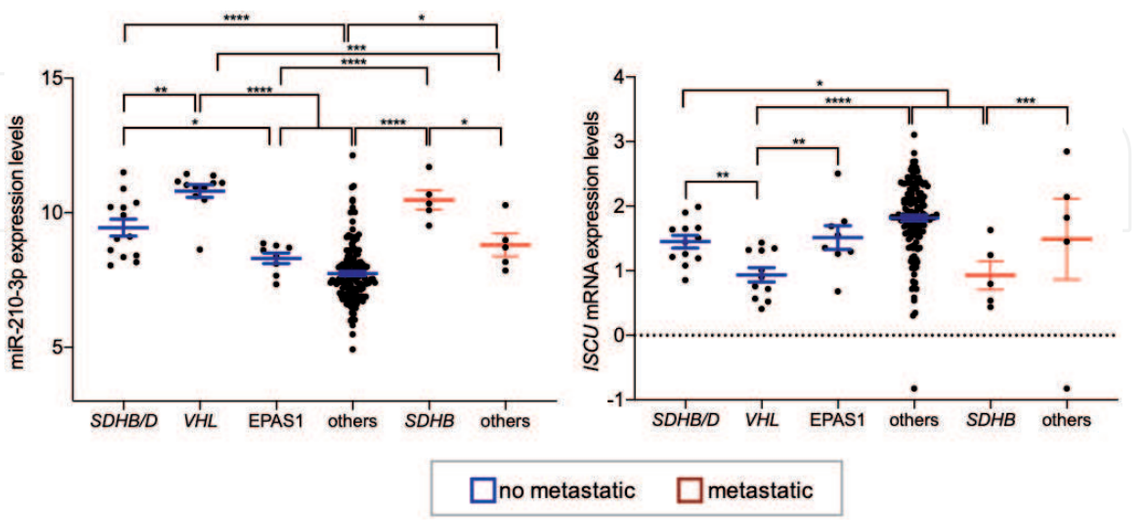

Figure 6.
*miR-210-ISCU signaling is moderately activated in SDHx-mutated PPGLs irrespective of their benign or metastatic behavior. miR-210-3p and ISCU levels in PPGLs included in the TCGA database are represented according to their genotype. Data from patients without or with metastasis are represented in blue and red, respectively. SDHB/D: PPGLs from patients with germline mutations in SDHB or SDHD genes; VHL: PPGLs with mutations in VHL; EPAS1: PPGLs with mutations in EPAS1; others: PPGLs with or without mutations in other PPGL-susceptibility genes; SDHB: metastatic PPGLs from patients with germline mutations in SDHB gene; **** P < 0.0001.*

lncRNAs are usually defined as non-coding RNAs greater than 200 nucleotides [70]. Although their functions are not well understood they seem to have key roles in gene regulation which depend on their localization and their specific interactions with DNA, RNA and proteins. Their tissue-specific and condition-specific expression patterns suggest that lncRNAs could be potential biomarkers. Recent reports described DGCR9, FENDRR, HIF1A-AS2, MIR210HG [71] and BC063866 [21] with significantly elevated expression in metastatic compared to benign PPGLs. Expression of BC063866 was found significantly elevated in *SDHx*-mutated metastatic PPGLs and, if validated in larger series, could be a novel biomarker to identify potentially metastatic tumors in patients carrying *SDHB* mutation.

5. Epigenetic drugs as therapeutic strategies for patients with metastatic PPGLs

Among epigenetic drugs, despite their limitations, DNA methyltransferase (DNMT) inhibitors are the most effective epigenetic therapy developed to date. Azacitidine and decitabine are cytidine analogues that incorporate themselves into replicating DNA and inhibit DNMTs. This implies that these inhibitors have broad cellular effects leading to global loss of DNA methylation. Hence their use as epigenetic drugs have to deal with strategies to minimize the off-target effects. The use of effective methods for drug delivery reduces side effects and attains a higher therapeutic index. There are various delivery systems like nanocarriers (nanogels, liposomes, dendrimers, and polymeric nanoparticles) that enhance drug stability, permeability and retention. Low doses have received regulatory approval for the treatment of myelodysplastic syndrome and acute myeloid leukemia who are not candidates for conventional induction chemotherapy. The use of the DNMT inhibitor, guadecitabine, is currently been evaluated in patients with PPGLs associated with SDH-deficiency under phase II clinical trial.

Other epigenetic drugs include the inhibitors of histone-lysine methyltransferases [72]. Multiple PRC2 inhibitors are currently being evaluated in ongoing phase I/II clinical trials in a range of cancers [73]. Most hypermethylated genes in metastatic *SDHB*-mutated PPGLs are PRC2 targets thus suggesting that patients could be benefited by the use of these epigenetic drugs [24].

The findings that overproduction of succinate suppresses HDR provide a mechanistic basis for the use novel effective strategies to exploit these defects for therapeutic gain. HDR repression in SDH-deficient tumors enhances cellular dependence on alternative, poly [ADP-ribose] polymerase (PARP) dependent DNA repair mechanisms, which appears to offer a compelling opportunity for targeted therapeutic intervention in oncometabolite-driven cancers. A large body of scientific evidence and clinical trials led to FDA approval of PARP inhibitor monotherapy for the treatment of various cancers harboring mutations in HDR machinery, including those with BRCA1/2 loss [74]. It should be explored whether the HDR defect conferred by succinate accumulation is strong enough to put into practice this therapeutic strategy in SDH-deficient driven cancers. One interesting possibility will be to add DNA-damaging therapies to PARP antagonists to maximize therapeutic efficacy. Notably, the PARP inhibitor olaparib in combination with temozolamide is currently undergoing testing in phase II clinical study in metastatic PPGLs.

Hypersuccinylation can also be a target of therapy in metastatic PPGLs. Succinyl-CoA accumulated in SDH-deficient tumors can be condensed with glycine by D-aminolevulinate synthase 1 to form 5-aminolevulinate and enter the heme biosynthesis pathway. Therefore, glycine supplementation may facilitate removal of succinyl-CoA and inhibit succinylation. Relief of hypersuccinylation by glycine

supplementation, has been shown to result in inhibited growth of hypersuccinylated tumors [59], thus shedding lights on alternative approaches for *SDHx*-mutated-PPGLs.

6. Conclusions

Metastasis is the most letal attribute of PPGLs, especially in patients with compromised SDH activity. Since the initial discovery of succinate as an oncometabolite that induces DNA hypermethylation, the knowledges that illustrate its role on epigenetic reprogramming and metastasis development continues to expand. The best characterized changes, DNA and histone methylation, could be efficiently and globally neutralized by DNA or histone hypomethylating agents, well-known epi-drugs that could be tested as single- or multi-drug therapy in metastatic SDH-deficient PPGLs. The activity of these epigenetic therapies, however, is not limited to cancer cells but have broad cellular effects leading to global loss of DNA methylation and off-target effects. Emerging scientific knowledges on the impacts that succinate-induced modification of the epigenetic code has on cancer development and progression is certainly empowering the research community to develop more effective, less toxic, and better tolerated therapies.

Acknowledgements

MDC is funded by Fondo de Investigación Sanitaria (grant numbers FIS PI17/01901 and PI20/01754) and the Red Temática de Investigación Cooperativa en Cáncer, CIBERONC, Instituto de Salud Carlos III (ISCIII).

Conflict of interest

There are not conflict of interest.

Notes/thanks/other declarations

L.C. thanks the Spanish Ministerio de Ciencia, Innovación y Universidades for the FPU predoctoral contract. T.C. thanks to the Red Temática de Investigación Cooperativa en Cáncer, CIBERONC, Instituto de Salud Carlos III (ISCIII) for her contract. A.S.J.M. thanks to Principado de Asturias and Fondo Europeo de Desarrollo Regional (GRUPIN) for his contract. **Figures 1–3** were created with BioRender.com.

Acronyms and abbreviations

DNMT	DNA methyltransferase
HDR	homology-dependent DNA repair
LRES	Long-range epigenetic silencing
PARP	poly [ADP-ribose] polymerase
PPGL	paraganglioma and pheochromocytoma
PRC2	polycomb repressive complex 2
SDH	succinate dehydrogenase
TCA	tricarboxylic acid cycle
TCGA	The cancer genome atlas

Author details

María-Dolores Chiara[1,2,3]*, Lucía Celada[1,3], Andrés San José Martinez[1], Tamara Cubiella[1,3], Enol Álvarez-González[1,2,4] and Nuria Valdés[1,5]

1 Institute of Sanitary Research of Principality of Asturias, Oviedo, Spain

2 Institute of Oncology of the Principality of Asturias, University of Oviedo, Oviedo, Spain

3 CIBERONC, Madrid, Spain

4 Department of Functional Biology, University of Oviedo, Oviedo, Spain

5 Section of Endocrinology and Nutrition, Hospital Universitario de Cabueñes, Gijón, Spain

*Address all correspondence to: mdchiara.uo@uniovi.es

References

[1] Buffet A, Burnichon N, Favier J, Gimenez-Roqueplo AP. An overview of 20 years of genetic studies in pheochromocytoma and paraganglioma. Best Practice and Research: Clinical Endocrinology and Metabolism. 2020;34(2):101416. DOI: 10.1016/j.beem.2020.101416

[2] Weber A, Hoffmann MM, Neumann HPH, Erlic Z. Somatic Mutation Analysis of the SDHB, SDHC, SDHD, and RET Genes in the Clinical Assessment of Sporadic and Hereditary Pheochromocytoma. Horm Cancer. 2012;3(4):187-192. DOI: 10.1007/s12672-012-0113-y

[3] Hamidi O. Metastatic pheochromocytoma and paraganglioma: recent advances in prognosis and management . Current Opinion in Endocrinology, Diabetes and Obesity. 2019;26(3):146-154. DOI: 10.1097/MED.0000000000000476

[4] Lam AK yin. Update on Adrenal Tumours in 2017 World Health Organization (WHO) of Endocrine Tumours . Endocrine Pathology. 2017; 28(3):213-227. DOI: 10.1007/s12022-017-9484-5

[5] Wachtel H, Hutchens T, Baraban E, Schwartz LE, Montone K, Baloch Z, et al. Predicting metastatic potential in pheochromocytoma and paraganglioma: A comparison of PASS and GAPP scoring systems. J Clin Endocrinol Metab . 2020;105(12). DOI: 10.1210/clinem/dgaa608

[6] Lee H, Jeong S, Yu Y, Kang J, Sun H, Rhee JK, et al. Risk of metastatic pheochromocytoma and paraganglioma in SDHx mutation carriers: A systematic review and updated meta-analysis. Journal of Medical Genetics. 2020;57(4):217-225. DOI: 10.1136/jmedgenet-2019-106324

[7] Crona J, Lamarca A, Ghosal S, Welin S, Skogseid B, Pacak K. Genotype-phenotype correlations in pheochromocytoma and paraganglioma: A systematic review and individual patient meta-analysis. Endocr Relat Cancer. 2019;26(5):539-550. DOI: 10.1530/ERC-19-0024

[8] Buffet A, Morin A, Castro-Vega L-J, Habarou F, Lussey-Lepoutre C, Letouzé E, et al. Germline Mutations in the Mitochondrial 2-Oxoglutarate/Malate Carrier SLC25A11 Gene Confer a Predisposition to Metastatic Paragangliomas. Cancer Res. 2018;78(8):1914-1922. DOI: 10.1158/0008-5472.can-17-2463

[9] Job S, Draskovic I, Burnichon N, Buffet A, Cros J ôme, Lepine C, et al. Telomerase activation and ATRX mutations are independent risk factors for metastatic pheochromocytoma and paraganglioma. Clin Cancer Res . 2019;25(2):760-770. DOI: 10.1158/1078-0432.CCR-18-0139

[10] Luo Z, Li J, Qin Y, Ma Y, Liang X, Xian J, et al. Differential expression of human telomerase catalytic subunit mRNA by in situ hybridization in pheochromocytomas. Endocr Pathol. 2006;17(4):387-398. DOI: 10.1007/s12022-006-0010-4

[11] Vezzosi D, Bouisson M, Escourrou G, Laurell H, Selves J, Seguin P, et al. Clinical utility of telomerase for the diagnosis of malignant well-differentiated endocrine tumours. Clin Endocrinol (Oxf). 2006;64(1):63-67. DOI: 10.1111/j.1365-2265.2005.02417.x

[12] Suh YJ, Choe JY, Park HJ. Malignancy in Pheochromocytoma or Paraganglioma: Integrative Analysis of 176 Cases in TCGA. Endocr Pathol. 2017;28(2):159-164. DOI: 10.1007/s12022-017-9479-2

[13] Fishbein L, Leshchiner I, Walter V, Danilova L, Robertson AG, Johnson AR, et al. Comprehensive Molecular Characterization of Pheochromocytoma and Paraganglioma. Cancer Cell. 2017;31(2):181-193. DOI: 10.1016/j.ccell.2017.01.001

[14] Letouzé E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, et al. SDH Mutations Establish a Hypermethylator Phenotype in Paraganglioma. Cancer Cell. 2013;23(6):739-752. DOI: 10.1016/j.ccr.2013.04.018

[15] Losman JA, Koivunen P, Kaelin WG. 2-Oxoglutarate-dependent dioxygenases in cancer. Nature Reviews Cancer. 2020;20(12):710-726. DOI: 10.1038/s41568-020-00303-3

[16] Pollard PJ, El-Bahrawy M, Poulsom R, Elia G, Killick P, Kelly G, et al. Expression of HIF-1α, HIF-2α (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. J Clin Endocrinol Metab. 2006;91(11):4593-4598. DOI: 10.1210/jc.2006-0920

[17] Bernardo-Castiñeira C, Sáenz-de-Santa-María I, Valdés N, Astudillo A, Balbín M, Pitiot AS, et al. Clinical significance and peculiarities of succinate dehydrogenase B and hypoxia inducible factor 1α expression in parasympathetic versus sympathetic paragangliomas. Head Neck. 2019;41(1):79-91. DOI: 10.1002/hed.25386

[18] Merlo A, de Quiros SB, Secades P, Zambrano I, Balbín M, Astudillo A, et al. Identification of a signaling axis HIF-1α/microRNA-210/ISCU independent of SDH mutation that defines a subgroup of head and neck paragangliomas. J Clin Endocrinol Metab. 2012;97(11):E2194-200. DOI: 97(11):E2194-200

[19] Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev. 2012;26(12):1326-1338. DOI: 10.1101/gad.191056.112

[20] Morin A, Goncalves J, Moog S, Castro-Vega LJ, Job S, Buffet A, et al. TET-Mediated Hypermethylation Primes SDH-Deficient Cells for HIF2α-Driven Mesenchymal Transition. Cell Rep. 2020;30(13):4551-4566.e7. DOI: 10.1016/j.celrep.2020.03.022

[21] Job S, Georges A, Burnichon N, Buffet A, Amar L, Bertherat J, et al. Transcriptome Analysis of lncRNAs in Pheochromocytomas and Paragangliomas. J Clin Endocrinol Metab. 2020;105(3):dgz168. DOI: 10.1210/clinem/dgz168

[22] Calsina B, Castro-Vega LJ, Torres-Pérez R, Inglada-Pérez L, Currás-Freixes M, Roldán-Romero JM, et al. Integrative multi-omics analysis identifies a prognostic miRNA signature and a targetable miR-21-3p/TSC2/ mTOR axis in metastatic pheochromocytoma/ paraganglioma. Theranostics. 2019;9(17):4946-4958. DOI: 10.7150/thno.35458

[23] Patterson E, Webb R, Weisbrod A, Bian B, He M, Zhang L, et al. The microRNA expression changes associated with malignancy and SDHB mutation in pheochromocytoma. Endocr Relat Cancer. 2012;19(2):157-166. DOI: 10.1530/ERC-11-0308

[24] Bernardo-Castiñeira C, Valdés N, Celada L, Martinez ASJ, Sáenz-de-Santa-María I, Bayón GF, et al. Epigenetic Deregulation of Protocadherin PCDHGC3 in Pheochromocytomas/Paragangliomas Associated With SDHB Mutations. J Clin Endocrinol Metab. 2019;104(11):5673-5692.

[25] Khan AA, Lee AJ, Roh TY. Polycomb group protein-mediated

histone modifications during cell differentiation. Epigenomics. Future Medicine. 2015; 7(1):75-84. DOI: 10.2217/epi.14.61

[26] Conway E, Healy E, Bracken AP. PRC2 mediated H3K27 methylations in cellular identity and cancer. Current Opinion in Cell Biology. 2015:37:42-48. DOI: 10.1016/j.ceb.2015.10.003

[27] Baksh SC, Finley LWS. Metabolic Coordination of Cell Fate by α-Ketoglutarate-Dependent Dioxygenases. Trends in Cell Biology. 2020;31(1):24-36DOI: 10.1016/j.tcb.2020.09.010

[28] Wu Q, Zhang T, Cheng JF, Kim Y, Grimwood J, Schmutz J, et al. Comparative DNA sequence analysis of mouse and human protocadherin gene clusters. Genome Research. 2001;11(3):389-404. DOI: 10.1101/gr.167301

[29] Mountoufaris G, Chen W V., Hirabayashi Y, O'Keeffe S, Chevee M, Nwakeze CL, et al. Multicluster Pcdh diversity is required for mouse olfactory neural circuit assembly. Science. 2017;356(6336):411-414. DOI: 10.1126/science.aai8801

[30] Thu CA, Chen W V., Rubinstein R, Chevee M, Wolcott HN, Felsovalyi KO, et al. Single-cell identity generated by combinatorial homophilic interactions between α, β, and γ protocadherins. Cell. 2014;158(5):1045-1059. DOI: 10.1016/j.cell.2014.07.012

[31] Chen W V., Maniatis T. Clustered protocadherins. De . 2013;140(16):3297-3302. DOI: 10.1242/dev.090621

[32] Chen W V., Alvarez FJ, Lefebvre JL, Friedman B, Nwakeze C, Geiman E, et al. Functional significance of isoform diversification in the protocadherin gamma gene cluster. Neuron. 2012;75(3):402-409. DOI: 10.1016/j.neuron.2012.06.039

[33] Lefebvre JL, Kostadinov D, Chen W V., Maniatis T, Sanes JR. Protocadherins mediate dendritic self-avoidance in the mammalian nervous system. Nature. 2012;488(7412):517-521. DOI: 10.1038/nature11305

[34] Wang KH, Lin CJ, Liu CJ, Liu DW, Huang RL, Ding DC, et al. Global methylation silencing of clustered proto-cadherin genes in cervical cancer: Serving as diagnostic markers comparable to HPV. Cancer Med. 2015;4(1):43-55. DOI: 10.1002/cam4.335

[35] Vega-Benedetti AF, Loi E, Moi L, Blois S, Fadda A, Antonelli M, et al. Clustered protocadherins methylation alterations in cancer. Clin Epigenetics. 2019;11(1):100. DOI: 10.1186/s13148-019-0695-0

[36] Banelli B, Brigati C, Di Vinci A, Casciano I, Forlani A, Borz L, et al. A pyrosequencing assay for the quantitative methylation analysis of the PCDHB gene cluster, the major factor in neuroblastoma methylator phenotype. Lab Investig. 2012;92(3):458-465. DOI: 10.1038/labinvest.2011.169

[37] Dallosso AR, Øster B, Greenhough A, Thorsen K, Curry TJ, Owen C, et al. Long-range epigenetic silencing of chromosome 5q31 protocadherins is involved in early and late stages of colorectal tumorigenesis through modulation of oncogenic pathways. Oncogene. 2012;31(40):4409-4419. DOI: 10.1038/onc.2011.609

[38] Vanharanta S, Massagué J. Origins of Metastatic Traits. Cancer Cell. 2013;24(4):410-421. DOI: 10.1016/j.ccr.2013.09.007

[39] Garrett AM, Bosch PJ, Steffen DM, Fuller LC, Marcucci CG, Koch AA, et al. CRISPR/Cas9 interrogation of the mouse Pcdhg gene cluster reveals a crucial isoform-specific role for

Pcdhgc4. PLoS Genet. 2019;15(12). DOI: 10.1371/journal.pgen.1008554

[40] Garrett AM, Schreiner D, Lobas MA, Weiner JA. γ-Protocadherins Control Cortical Dendrite Arborization by Regulating the Activity of a FAK/PKC/MARCKS Signaling Pathway. Neuron. 2012;74(2):269-276. DOI: 10.1016/j.neuron.2012.01.028

[41] Chen J, Lu Y, Meng S, Han MH, Lin C, Wang X. α- and γ-protocadherins negatively regulate PYK2. J Biol Chem. 2009;284(5):2880-2890. DOI: 10.1074/jbc.M807417200

[42] Suo L, Lu H, Ying G, Capecchi MR, Wu Q. Protocadherin clusters and cell adhesion kinase regulate dendrite complexity through Rho GTPase. J Mol Cell Biol. 2012;4(6):362-376. DOI: 10.1093/jmcb/mjs034

[43] Keeler AB, Schreiner D, Weiner JA. Protein kinase C phosphorylation of a γ-protocadherin C-terminal lipid binding domain regulates focal adhesion kinase inhibition and dendrite arborization. J Biol Chem. 2015;290(34):20674-20686. DOI: 10.1074/jbc.M115.642306

[44] Keeler AB, Molumby MJ, Weiner JA. Protocadherins branch out: Multiple roles in dendrite development . Cell Adhesion and Migration. 2015;9(3):214-226. DOI: 10.1080/19336918.2014.1000069

[45] Oudijk L, Papathomas T, De Krijger R, Korpershoek E, Gimenez-Roqueplo AP, Favier J, et al. The mTORC1 Complex Is Significantly Overactivated in SDHX -Mutated Paragangliomas. Neuroendocrinology. 2017;105(4):384-393. DOI: 10.1159/000455864

[46] Zhang X, Wang X, Qin L, Xu T, Zhu Z, Zhong S, et al. The dual mTORC1 and mTORC2 inhibitor PP242 shows strong antitumor activity in a pheochromocytoma PC12 cell tumor model. Urology. 2015;85(1):273.e1-273.e7. DOI: 10.1016/j.urology.2014.09.020

[47] Cervera AM, Bayley JP, Devilee P, McCreath KJ. Inhibition of succinate dehydrogenase dysregulates histone modification in mammalian cells. Mol Cancer. 2009;8:89. DOI: 10.1186/1476-4598-8-89

[48] Smith EH, Janknecht R, Maher JL. Succinate inhibition of α-ketoglutarate-dependent enzymes in a yeast model of paraganglioma. Hum Mol Genet. 2007;16(24):3136-3148. DOI: 10.1093/hmg/ddm275

[49] Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. Experimental and Molecular Medicine. 2017;49(4):e324. DOI: 10.1038/emm.2017.11

[50] Ljungman M, Parks L, Hulbatte R, Bedi K. The role of H3K79 methylation in transcription and the DNA damage response. Mutation Research. 2019;780:48-54. DOI: 10.1016/j.mrrev.2017.11.001

[51] Sulkowski PL, Oeck S, Dow J, Economos NG, Mirfakhraie L, Liu Y, et al. Oncometabolites suppress DNA repair by disrupting local chromatin signalling. Nature. 2020;582(7813):586-591. DOI: 10.1038/s41586-020-2363-0

[52] Savani MR, Abdullah KG, McBrayer SK. Amplifying the Noise: Oncometabolites Mask an Epigenetic Signal of DNA Damage. Mol Cell . 2020;79(3):368-370. DOI: 10.1016/j.molcel.2020.07.014

[53] Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature. 2016;529(7584):110-114. DOI: 10.1038/nature16490

[54] Özdemir I, Gambetta MC. The role of insulation in patterning gene

expression. Genes. 2019;10(10):767. DOI: 10.3390/genes10100767

[55] Flavahan WA, Drier Y, Johnstone SE, Hemming ML, Tarjan DR, Hegazi E, et al. Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. Nature. 2019;575(7781):229-233. DOI: 10.1038/s41586-019-1668-3

[56] Zhao Y, Feng F, Guo QH, Wang YP, Zhao R. Role of succinate dehydrogenase deficiency and oncometabolites in gastrointestinal stromal tumors. World Journal of Gastroenterology. 2020;26(34):5074-5089. DOI: 10.3748/WJG.V26.I34.5074

[57] Zhang Z, Tan M, Xie Z, Dai L, Chen Y, Zhao Y. Identification of lysine succinylation as a new post-translational modification. Nat Chem Biol . 2011;7(1):58-63. DOI: 10.1038/nchembio.495

[58] Li F, He X, Ye D, Lin Y, Yu H, Yao C, et al. NADP+-IDH Mutations Promote Hypersuccinylation that Impairs Mitochondria Respiration and Induces Apoptosis Resistance. Mol Cell. 2015;60(4):661-675. DOI: 10.1016/j.molcel.2015.10.017

[59] Wang Y, Guo YR, Liu K, Yin Z, Liu R, Xia Y, et al. KAT2A coupled with the α-KGDH complex acts as a histone H3 succinyltransferase. Nature. 2017;552(7684):273-277. DOI: 10.1038/nature25003

[60] Smestad J, Erber L, Chen Y, Maher LJ. Chromatin Succinylation Correlates with Active Gene Expression and Is Perturbed by Defective TCA Cycle Metabolism. iScience. 2018;2:63-75. DOI: 10.1016/j.isci.2018.03.012

[61] Guo Z, Pan F, Peng L, Tian S, Jiao J, Liao L, et al. Systematic Proteome and Lysine Succinylome Analysis Reveals the Enhanced Cell Migration by Hyposuccinylation in Esophageal Squamous Cell Cancer. Mol Cell Proteomics . 2020;mcp.RA120.002150. DOI: 10.1074/mcp.ra120.002150

[62] Yang G, Yuan Y, Yuan H, Wang J, Yun H, Geng Y, et al. Histone acetyltransferase 1 is a succinyltransferase for histones and non-histones and promotes tumorigenesis. EMBO Rep. 2020;e50967. DOI: 10.15252/embr.202050967

[63] Castro-Vega LJ, Letouzé E, Burnichon N, Buffet A, Disderot PH, Khalifa E, et al. Multi-omics analysis defines core genomic alterations in pheochromocytomas and paragangliomas. Nat Commun. 2015;6:6044. DOI: 10.1038/ncomms7044

[64] Meyer-Rochow GY, Jackson NE, Conaglen J V., Whittle DE, Kunnimalaiyaan M, Chen H, et al. MicroRNA profiling of benign and malignant pheochromocytomas identifies novel diagnostic and therapeutic targets. Endocr Relat Cancer. 2010;17(3):835-846. DOI: 10.1677/ERC-10-0142

[65] Bavelloni A, Ramazzotti G, Poli A, Piazzi M, Focaccia E, Blalock W, et al. Mirna-210: A current overview. Anticancer Research. 2017;37(12):6511-6521. DOI: 10.21873/anticanres.12107

[66] Tsang VHM, Dwight T, Benn DE, Meyer-Rochow GY, Gill AJ, Sywak M, et al. Overexpression of miR-210 is associated with SDH-related pheochromocytomas, paragangliomas, and gastrointestinal stromal tumours. Endocr Relat Cancer. 2014;21(3):415-426. DOI: 10.1530/ERC-13-0519

[67] Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 Controls Mitochondrial Metabolism during Hypoxia by Repressing the Iron-Sulfur Cluster Assembly Proteins ISCU1/2. Cell Metab. 2009;10(4):273-284. DOI: 10.1016/j.cmet.2009.08.015

[68] Ruff SM, Ayabe RI, Malekzadeh P, Good ML, Wach MM, Gonzales MK, et al. MicroRNA-210 May Be a Preoperative Biomarker of Malignant Pheochromocytomas and Paragangliomas. J Surg Res. 2019;243:1-7. DOI: 10.1016/j.jss.2019.04.086

[69] Merlo A, Bernardo-Castiñeira C, Sáenz-de-Santa-María I, Pitiot AS, Balbín M, Astudillo A, et al. Role of VHL, HIF1A and SDH on the expression of miR-210: Implications for tumoral pseudo-hypoxic fate. Oncotarget. 2016;8(4):6700-6717. DOI: 10.18632/oncotarget.14265

[70] Ponting CP, Oliver PL, Reik W. Evolution and Functions of Long Noncoding RNAs. Cell. 2009;136(4):629-641. DOI: 10.1016/j.cell.2009.02.006

[71] Ghosal S, Das S, Pang Y, Gonzales MK, Huynh TT, Yang Y, et al. Long intergenic noncoding RNA profiles of pheochromocytoma and paraganglioma: A novel prognostic biomarker. Int J Cancer. 2020;146(8):2326-2335. DOI: 10.1002/ijc.32654

[72] Rugo HS, Jacobs I, Sharma S, Scappaticci F, Paul TA, Jensen-Pergakes K, et al. The Promise for Histone Methyltransferase Inhibitors for Epigenetic Therapy in Clinical Oncology: A Narrative Review. Advances in Therapy. 2020; 37(7):3059-3082 p. 3059-82. DOI: 10.1007/s12325-020-01379-x

[73] Duan R, Du W, Guo W. EZH2: A novel target for cancer treatment. Journal of Hematology and Oncology. 2020;13(1):104. DOI: 10.1186/s13045-020-00937-8

[74] Slade D. PARP and PARG inhibitors in cancer treatment . Genes and Development. 2020;34 (5-6):360-394 p. 360-94. DOI: 10.1101/gad.334516.119