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Genetic and epigenetic differences of benign and malignant pheochromocytomas and paragangliomas (PPGLs)

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Pheochromocytomas and paragangliomas (PPGLs) are tumors arising from the adrenal medulla and sympathetic/parasympathetic paraganglia, respectively. According to The Cancer Genome Atlas (TCGA), approximately 40% of PPGLs are due to germ line mutations in one of 16 susceptibility genes, and a further 30% are due to somatic alterations in at least seven main genes (*VHL*, *EPAS1*, *CSDE1*, *MAX*, *HRAS*, *NF1*, *RET*, and possibly *KIF1B*). The diagnosis of malignant PPGL was straight forward in most cases as it was defined as presence of PPGL in non-chromaffin tissues. Accordingly, there is an extreme need for new diagnostic marker(s) to identify tumors with malignant prospective. The aim of this study was to review all suggested genetic and epigenetic alterations that are remarkably different between benign and malignant PPGLs. It seems that more than two genetic mutation clusters in PPGLs and other genetic and methylation biomarkers could be targeted for malignancy discrimination in different studies.

Key words: pheochromocytoma, paraganglioma, genetic, epigenetic, methylation

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors which arise in the sympathetic and parasympathetic paraganglia (Boron and Boulpaep 2009). According to WHO definition "intra-adrenal paraganglioma, highlighting the common origin of phaeochromocytomas and sympathetic or parasympathetic paragangliomas, which are all derived from neuroectoderm and can all occur in patients with the same genetic predisposition" (DeLellis et al. 2004; Favier et al. 2015). The majority of PPGLs are sporadic and benign with incidence rate of about 0.4–9.5 per 10^6 people (Stenstrom and Svardsudd 1986; Larijani et al. 2004), produce catecholamine's, nor epinephrine and/or epinephrine as PPGL may produce a combination or be strictly noradrenergic (Eisenhofer et al. 2004a). According to grading system for adrenal phaeochromocytoma and paraganglioma (GAPP), the tumors are scored based on histological pattern, cellularity, comedo-type necrosis, capsular/vascular invasion, *Ki67* labeling index, and catecholamine type and are graded as one of the three types: well-differentiated (WD, 0–2 points), moderately differentiated (MD, 3–6 points) and poorly differentiated (PD, 7–10 points) (Kimura et al. 2014).The morbidity and mortality of these tumors are related to its aggressive behavior, metastasis, and effective role of catecholamine's on different organs like cardiovascular system (Capelli et al. 2009, Yalcin and Oberg 2015). The tumor is malignant in 10% of cases and unfortunately is not curable completely by surgical removal. Many tumors of PPGLs remain unrecognized and are diagnosed only during

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postmortem examination (McNeil et al. 2000). Measurements of plasma methoxytyramine, the O-methylated dopamine metabolite, are useful for detecting rare dopamine-producing PPGLs and head and neck paragangliomas (HNPGLs) (Rao et al. 2017). Unfortunately, it has not been a comprehensive approach in diagnosis and treatment of malignant PPGLs yet (Thompson 2002).

Because PPGLs have been demonstrated to exhibit intra- and inter-tumor heterogeneity in terms of biology, there is a real chance that location bias could be incorporated in most of the studies conducted so far. However, some histological features have been described as diagnostic or predicting tools in different tumors (Kleihues et al. 2002; Tavangar et al. 2004). In malignant PPGLs, some features including necrosis, vascular invasion, and extensive capsular invasion have been reported to correlate with malignancy (Linnoila et al. 1990; van der Harst et al. 2000). Prognostic value of PCCs of the Adrenal Gland Scaled Score (Pass score) tests to separate benign from malignant neoplasms has been introduced by Thompson et al. (2002). Several histological features have been taken into consideration including growth pattern, necrosis, local and metastatic invasion, nuclear pleomorphic, cellularity and cellular monotony, tumor cell spindling, mitotic count and atypical mitotic figures (Thompson 2002). Paying inadequate consideration to how blood is collected and how results are interpreted influences the high rates of misdiagnosis and major problem that comes from this inability to identify metastatic PPGL is that all cases with R0 resection needs long follow-up and (Yu et al. 2009; Chen et al. 2010; Guo and Lloyd 2015).

Thanks to recent advances in genetic science, some mutations with various consequences like protein expression, immune-reactivity alongside with epigenetic alterations, have been considered as appropriate discriminative markers for malignancy in different tumors (Tavangar et al. 2005; Haghpanah et al. 2006; Tavangar et al. 2007; Sarmadi et al. 2009; Tabriz et al. 2009; Mohammadi-asl et al. 2011; Sanii et al. 2012). It has been reported that up to 40% of patients with PPGLs have a germ line mutation in recognized susceptibility genes (Mannelli et al. 2009; Fishbein et al. 2013; Favier et al. 2015), the exact molecular differentiation between benign and malignant PPGLs is not understood at all. In this review, we provide an overview to the current data on genetic and epigenetic alterations of PPGLs as well as diversity of these variations between benign and malignant tumors.

Genetic mutations clustering in PPGLs

PPGLs have been suggested that are usually associated with 3 syndromes – *Von Hippel-Lindau (VHL)* syndrome (Hasani-Ranjbar et al. 2009), multiple endocrine neoplasia type 2 *(MEN 2;* Hasani-Ranjbar et al. 2011), and neurofibromatosis type 1 *(NF1*). Around 40% of PPGLs patients have a germ line mutation in one of the 16 famous susceptibility genes: *RET, NF1, VHL*, succinate dehydrogenases *(SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2*), *TMEM127*, *PHD1*, *PHD2, HIF2A*, *FH*, *Myc-associated factor (MAX)*, and *KIF1B* (Bayley et al. 2010; Majidi et al. 2011; Burnichon et al. 2012; Darr et al. 2012; Galan and Kann 2013; Letouze et al. 2013; Castro-Vega et al. 2014; Dahia 2014; Welander et al. 2014; Yang et al. 2015). In fact, there are two separate gene clusters taking part in the tumor genesis of PPGLs according to their transcriptional profile; kinase receptor-signaling gene cluster (associated with *RET/NF1/ TMEM127/MAX/ KIF1B* mutations) and a pseudo-hypoxic gene cluster (associated with mutations in *VHL/SDHx/PHD2* genes) (Nolting and Grossman 2012). According to the signaling pathways and transcriptome studies, all candidate genes for PPGLs are grouped in two clusters that seem to show separate and discrete routes for tumor genesis.

The first group (Group I), there are *VHL*, *SDH*, and prolyl hydroxylase domain *PHD* genes as well as markers of pseudohypoxia (*EPAS1*, *NOX4*, *LOXL2*), angiogenesis factors like (vaso-endothelial growth factor, *VEGF*) (Gimenez-Roqueplo 2004), and reduced oxidative response (Baysal et al. 2002; Neumann et al. 2002; Bryant et al. 2003; Dahia et al. 2005; Nolting and Grossman 2012; Zhikrivetskaya et al. 2017). The second group (Group II) includes the *RET*, *NF1*, trans-membrane protein 127 (*TMEM127*), kinesin family member 1B-beta isoform (*KIF1Bβ*), and *MAX* genes (Zhikrivetskaya et al. 2017). Additionally, tumors of this group are considered to by the diminished regulation of several signaling pathways *[PI3K/AKT*, *RAS/RAF/ERK*, and *mTORC1/p70S6* kinase (*p70S6K)]* besides translation initiation, protein synthesis, and neuronal (*SHANK2* and *RET*) plus neuroendocrine (*PNMT*, *NCAM2*, and *CADPS*) differentiation (Kajbafzadeh et al. 2006; Salajegheh et al. 2011; Nolting and Grossman 2012; Salajegheh et al. 2013; Zhikrivetskaya et al. 2017).

As it is shown in Figure 1, in the normal state group 1 gene contributes to the hypoxia response, so these gene mutations cause activation of effecter molecules in the absence of hypoxia. Group I tumors show a bigger rate of angiogenesis and over expres-

sion of *VEGF* and its receptors (Favier and Gimenez-Roqueplo 2010, 2012).The second set (cluster II) comprises of the proto-oncogene *RET* (which encodes a receptor tyrosine kinase) or the tumor suppressor genes (*NF1, TMEM127, MAX*, or *KIF1Bβ*) and related kinase signaling pathways; for example, *PI3Kinase/AKT* and *mTOR* (Figure 2) (Favier and Gimenez-Roqueplo 2010, 2012; Gimenez-Roqueplo et al. 2012; Zhikrivetskaya et al. 2017). The second set (cluster 2) comprises of the proto-oncogene *RET* (which encodes a receptor tyrosine kinase) or the tumor suppressor genes *NF1*, *TMEM127*, *MAX*, or *KIF1Bβ* and related kinase signaling pathways (Amar et al. 2005; Comino-Mendez et al. 2011; Shuch et al. 2014). This cluster, which is a kinase signaling cluster, involves genetic mutations associated with abnormal stimulation of kinase signaling pathways such as *PI3Kinase/AKT*, and the *mTOR* pathway (Gimenez-Roqueplo et al. 2012). In Figure 2, the molecular pathway of these genes and downstream targets are shown.

These two clusters are additionally subdivided according to their transcription

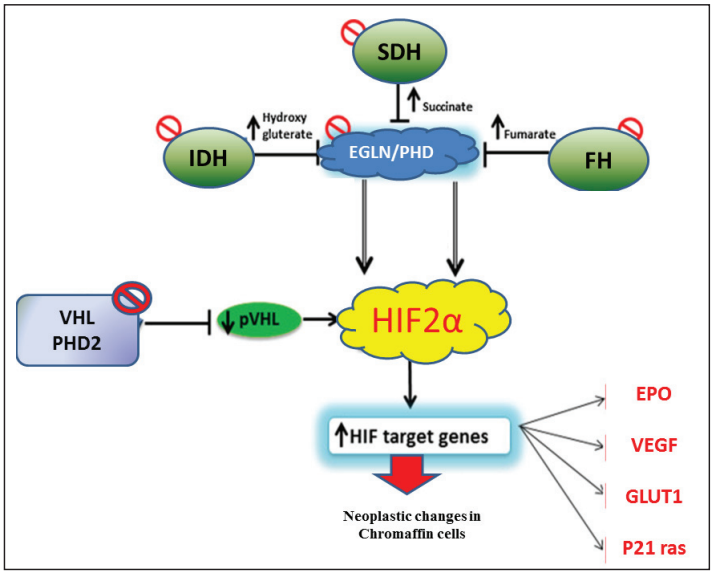

Figure 1. Impaired hypoxic status regulation owing to mutations in Group 1 genes (genes involved in the pseudohypoxic pathway of tumor development). Mutations in *VHL*, *SDH*, *HIF2A*, *PHD2*, and *FH* genes (pink color) may lead to activation of the transcription factor *HIF-1* and its target genes that promote pseudo hypoxic oncogenes (Zhikrivetskaya et al. 2017). In the normal state, these genes participate in the response to hypoxia; conversely, mutations impair the regulation of this response, principal to the activation of effectors molecules in the absence of hypoxia. Group I tumors display an increased rate of angiogenesis and elevated expression of VEGF and its receptors. Remarkably, these elevated expression levels have been observed in both benign and malignant paragangliomas.

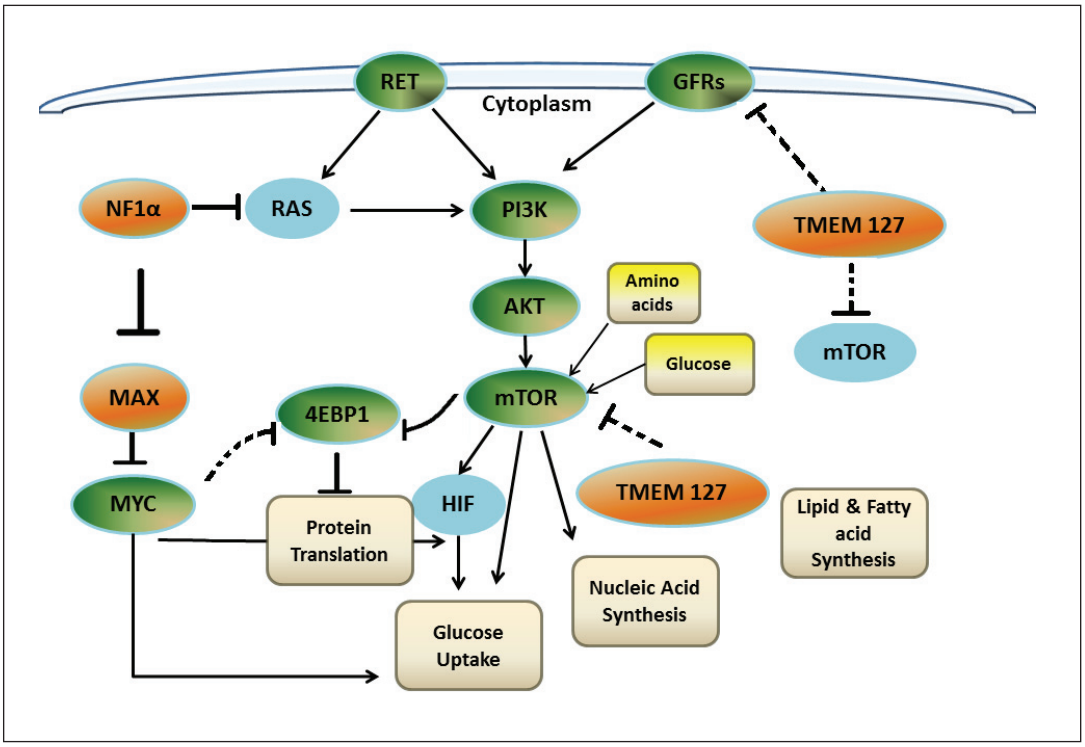

Figure 2. Impaired growth factor signaling owing to mutations in Group 2 genes (Zhikrivetskaya et al. 2017).

profiles (Pillai et al. 2016) (Figure 3). Cluster 1 could be distributed into sub cluster 1A and 1B, cluster 1A contains PPGLS related to *SDHx* and *FH*, while Cluster 1B contains tumors with *HIF2A* and *VHL*, respectively (Lopez-Jimenez et al. 2010). Cluster 2 can be divided into groups 2A, 2B, 2C, and 2D. Group 2A is consisting of *RET, MAX, NF1* and *TMEM127* mutated tumors but groups 2B and 2C are sporadic tumors (Dahia et al. 2005). Group 2D tumors carry unknown mutations related to PPGLs (Figure 3).

The genes VHL, SDHX from Group I and *RET*, and NF1 from Group II are highly required for apoptosis in neuronal precursor cells. The c-Jun protein is activated in the absence of signal from nerve growth factor (NGF) and causes neuronal cell apoptosis (Palmada et al. 2002). In the absence of neurofibromin the NF1 gene product prevents the NGF receptor *TrkA* and the embryonic sympathetic neurons survive even without the NGF signal (Vogel et al. 1995; Palmada et al. 2002). It has been shown that in the pheochromocytoma-derived cell line PC12, the succinate increase made cell growth through inhibiting PHD3-dependent apoptosis, which directed to the embryonic neurons survival and the PPGLs development (Lee et al. 2005). VHL protein inactivation leads to Jun-B mutation (an antagonist of c-Jun) (Lee et al. 2005). The Prolyl hydroxylase 3 (PHD3) protein is necessary and sufficient for apoptosis induction after the termination of the NGF signal; consequently, damaging this protein function or impairment of its regulation through succinate accumulation, inhibits apoptosis and resulting in tumor formation (Lee et al. 2005; Zhikrivetskaya et al. 2017). Moreover, the contribution of menin protein, which is coded by multiple endocrine neoplasia type 1 (MEN1) in c-Jun activation and its suppression by the MYC protein propose that mutations in MEN1 and MAX can play a crucial role in PPGLs development (Agarwal et al. 1999; Vaque et al. 2008; Akerstrom 2016). Interestingly, a blood-based MAAA (NETest) has newly been industrialized for neuroendocrine tumors (NETs) (Li et al. 2013; Walenkamp et al. 2014; Kidd et al. 2015; Peczkowska et al. 2017) and confirmed to have efficacy in identifying residual disease (Modlin et al. 2016), describing progression (Pavel et al. 2017) and predicting treatment efficacy (Cwikla et al. 2015).

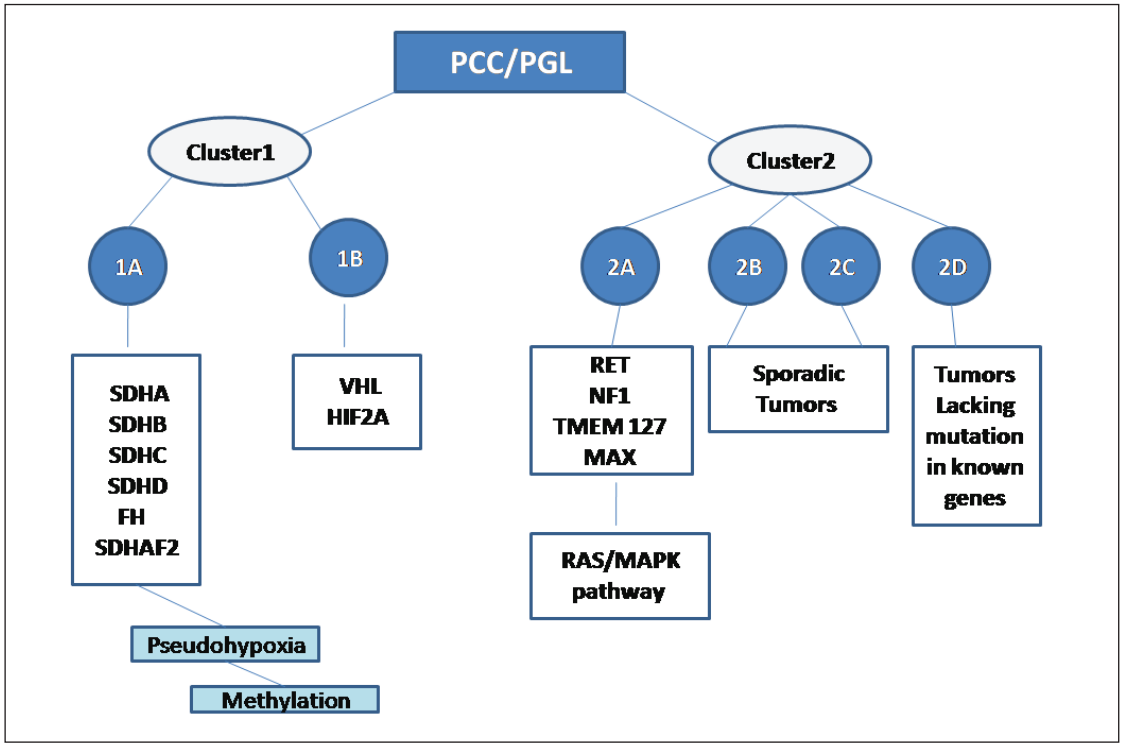

Figure 3. The subdivisions of cluster 1 and 2 genes in PPGLs accordingly to tumor biology. Cluster 1 genes could be divided into 2 groups. Cluster 1A contains *SDHx* and *FH*, while Cluster 1B contains tumors with *HIF2A* and *VHL*, respectively. Cluster 2 can be divided into subcluster 2A, 2B, 2C and 2D. Subcluster 2A comprises *RET*, *MAX*, *NF1* and *TMEM127* mutated tumors, whereas subcluster 2B and 2C are sporadic tumors. Cluster 2D are tumors lacking known mutations related to PPGLs (Pillai et al. 2016).

Genetic differences between benign and malignant PPGLs

Diagnosis of malignant "PPGLs", which presents about 10–15% of cases, is still under debate. Histological perception from benign cases is untrustworthy, so there is no definite diagnosis or cure for metastatic courses (Eisenhofer et al. 2004b). The exact molecular distinction of benign and malignant PPGLs could be a valuable development. In this respect, some tissue specific indicators for malignancy have been characterized, including different angiogenetic factors (Favier et al. 2002; Salmenkivi et al. 2003), cyclooxygenase-2 (Salmenkivi et al. 2001), secretogranin II-derived peptide (Yon et al. 2003). The 90 kDa heat shock protein (Hsp90) as a key for telomerase activation and malignant transition in PPGLs have been described as a possible therapeutic target in advanced PPGLs (Giubellino et al. 2013). Moreover, overexpression of human telomerase reverse transcriptase (*hTERT*) and related high telomerase activity (Boltze et al. 2003) or high *Ki-67/MIB-1* immuno-reactivity (Elder et al. 2003; Tavangar et al. 2010) could be the indicator of the invasive behavior of tumor with remarkable specificity (Elder et al. 2003). Tumor size and the histomorphologic patterns (spindle cell and small round cell) have been expressively related to the tumor behavior, *Ki-67* positivity and *c-erbB-2* expression were suggested as immunohistochemical markers for predicting the malignant behavior of PPGLs (Nasseri-Moghaddam et al. 2003; Tavangar et al. 2010).Transformed *hsp90* and hTERT not only are valuable for the classification of PPGLs as malignant, but could also be beneficial for choosing the best therapeutic policy, because cancer cells are mainly dependent on hsp90 to guarantee precise folding and function of mutated and overexpressed oncoproteins. In fact telomerase catalytic subunit (*hTERT*) activity and Hsp90 expression possibly will act as molecular markers for early diagnosis of malignancy, so before the surgery of familial syndromes which are considered both in benign and malignant PPGLs, testing *HSP90* and *hTERT* is highly demanded (Salmenkivi et al. 2004; Scholz et al. 2007). Contradictory, it has been shown that SDHB status and primary tumor size were more predictive of patient outcome than *Ki-67* (Assadipour et al. 2017). SDHB mutations in patients with malignant PPGLs were linked to the shorter survival so it has prognostic value for malignancy specially for patients with an apparent sporadic and/or benign presentation at diagnosis (Amar et al. 2007). Germ line *SDHB* mutations have also been associated with renal cell carcinoma, gastrointestinal stromal tumors, and papillary thyroid carcinoma (Mulukutla et al. 2016).

The *GATA3* belongs to the *GATA* family of transcription factors that regulate luminal epithelial cell differentiation in the mammary gland and plays fundamental roles in cell-fate specification (Kouros-Mehr et al. 2006). The staining pattern of *GATA3* showed that as a part of a panel of immunohistochemical markers, *GATA3* was beneficial for differential diagnosis (Perrino et al. 2017). Moreover, down-regulation of six metastasis suppressor genes in malignant PPGLs had been shown using quantitative real-time polymerase chain reaction (qRT-PCR) (Ohta et al. 2005). These metastasis suppressor genes are *nm23-H1*, *TIMP-4*, *BRMS-1*, *TXNIP*, *CRSP-3* and *E-Cad* that were down-regulated considerably in malignant PPGLs (Ohta et al. 2005; Saffar et al. 2011). Negative *nm-23*, laterally with positive *COX-2* or *galectin-3* indicated presence of malignancy and dual negativity for *galectin-3* and *COX-2*, alongside with positive nm-23 specified benign behavior (Saffar et al. 2011). Erythroblast leukemia viral oncogene homolog 2 (*ERBB-2*) encodes a member of the epidermal growth factor (*EGF*) receptor family of receptor tyrosine kinases. The over expression of *ERBB-2*, as an early event of tumor genesis, was more observed in malignant than benign PPGLs by multiplex ligation-dependent probe amplification and immunohistochemistry (Yuan et al. 2008). The microarray analysis of differentially expressed genes uncovered the lower expression of three key genes encoding peptide processing and activation factors including peptidyl glycine α amidating monooxygenase (*PAM*), glutaminyl-peptide cyclotransferase (*QPCT*) and neuropeptide Y (*NPY*) in malignant PPGLs (Helman et al. 1989; Thouennon et al. 2007). An assessment of the data of PPGLs from 90 patients, including 20 with malignant tumors identified, a total of 636 genes that discriminated benign from malignant disease at a significance level (Brouwers et al. 2006).The expression of 70% of these genes were reduced in malignant compared to benign tumors (Brouwers et al. 2006), proposing that malignant potential is largely characterized by a less-differentiated pattern of gene expression (Brouwers et al. 2006). Fishbein et al. (2016, 2017) described a comprehensive molecular characterization of PPGLs which are driven by diverse alterations affecting multiple genes and pathways. According to The Cancer Genome Atlas (TCGA), Cold Shock Domain-containing E1 gene (*CSDE1*) mutations resulted in decreased expression and may lead to inhibition of apoptosis, or programmed cell death. By diminishing apoptosis, *CSDE1* mutations may prevent the cell's normal

process of self-destruction (Fishbein et al. 2017). In fact, *CSDE1* was a somatically mutated driver gene, accompanying four known drivers (*HRAS*, *RET*, *EPAS1*, and *NF1*). Furthermore, fusion genes in PPGLs, involving Mastermind like Transcriptional Coactivator 3 (MAML3), *BRAF*, *NGFR*, and *NF1* were explored (Fishbein et al. 2017) but no dissimilarities between malignant and benign have been described. Unique *MAML3* fusion genes spanned three isoforms and were triggering based on overexpression of *MAML3* and on fusion transcript exonic expression (Fishbein 2016). The association between *MAML3* overexpression and activation of the *Wnt* signaling pathway has been shown resulting to bigger cell proliferation and metastasis (Fishbein 2016). Suggesting three molecular markers were positively linked with clinically aggressive disease: germline mutations in *SDHB*, somatic mutations in *ATRX*, a *SWI/SNF* chromatin remodeling protein (Fishbein et al. 2015), and fusions involving *MAML3* (Fishbein 2016).

Circulating tumor DNA and cells are one of the newest topics in cancer diagnosis policies (Khatami et al. 2017a,b). To assess whether a 51-neuroendocrine gene in blood analysis has clinical value as a diagnostic and prognostic marker circulating neuroendocrine tumor mRNA measured had been checked by qPCR analysis and positive circulating NET transcript analysis in well-differentiated PPGLs was shown (Peczkowska et al. 2017). Cell cycle arrest/release proteins, like *P53, Bcl-2, mdm-2, cyclinD1, p21*, and *p27*, appear to have no role in foretelling the behavior of PPGLs contrary to *Ki*-67 which is really beneficial risk for recurrence risk assessment (Strong et al. 2008). The expression of pituitary tumor transforming gene (*PTTG1*), an anaphase-promoting complex (*APC*) substrate, suggested as a potential marker for distinguishing benign versus malignant tumors (Amousha et al. 2015). A pair-wise (tumor-normal) whole-exome sequencing to analyze the somatic mutational landscape of malignant PPGLs pointed out mutations in transport and cell adhesion genes, like N-myc proto-oncogene protein (*MYCN*), myosin VB (*MYO5B*) and vinculin (*VCL*) (Wilzen et al. 2016). It was established that *MYO5B* mutation is indicator of malignancy, so deregulation of *Rab* and *Rac/Rho* pathways could play significant role in PPGLs tumor genesis (Wilzen et al. 2016). Lysine methyltransferase 2D (*KMT2D)* is a histone methylatransferase and a component of a large protein complex called *ASCOM* (transcriptional regulator of the *beta-globin* and *estrogen receptor* genes). It has been indicated that *KMT2D* expression was up regulated in PCCs compared to normal adrenals and its over expression certainly boosted cell migration in a PCC cell line (Juhlin et al. 2015). More than that a study disclosed a new function for FH in susceptibility to malignant and/or multiple PPGLs. Surprisingly, FH-deficient PPGLSs show the same pattern of epigenetic deregulation as SDHB-mutated malignant PPGLs. Then, they suggested that FH mutation screening could be included in PPGLS genetic testing, especially for tumors with malignant behavior (Castro-Vega et al. 2014). PPGLSs with somatic *ATRX* mutations are coupled with alternative lengthening of telomeres and clinically aggressive behavior because loss of *ATRX*, a SWI/SNF chromatin remodeling protein, is extremely essential in the development of clinically aggressive PPGLs (Fishbein et al. 2015). In Table 1, genetic alteration and resulting protein product changes, which were evidence for meaningful differences in malignant PPGLs are summarized.

Epigenetic differences between benign and malignant pheochromocytoma

Epigenetic changes are essential in molecular oncology because they affect gene transcription without changing the sequence of DNA. Aberrant DNA methylation has been known as one of the main features of human cancers (Jones 1986; Khatami et al. 2009a,b). Early studies were based on sequencing and PCR based methods to investigate the methylation status of carefully selected candidate genes (Geli et al. 2007; Muscarella et al. 2008), whereas latest studies have used array based on technologies to explore genome wide methylation (Letouze et al. 2013; de Cubas et al. 2015; Backman et al. 2017). The methylation level of some CpG-sites was projected as indicator of malignancy in PPGLs (de Cubas et al. 2015). Epigenetic profiling of PPGLs has been done to evaluate promoter CpG-methylation of some candidate genes (Geli et al. 2007; Margetts et al. 2008). Differentially methylated genes of normal tissue, benign tumors, and malignant tumors suggested that abnormal DNA methylation influence genes involved in cell cycle regulation and tumor morphology (Backman et al. 2015). The integrated analysis of the tumor expression made clear that there is a definite association between histone methylations and DNA copy number alterations on global gene transcription (Sandgren et al. 2010). Global DNA methylation analysis acknowledged two distinct clusters of tumors characterized by different methylation patterns and different driver mutations (Backman et al. 2017).

Remarkably, several candidate tumor suppressor genes are recognized with decreased expression like

TGIF1, *DSC3*, *TNFRSF10B*, *RASSF2*, *HOXA9*, *PTPRE* and *CDH11* and some other genes with increased expression including *GNAS*, *INSM1*, *DOK5*, *ETV1*, *RET*, *NTRK1*, *IGF2* (Sandgren et al. 2010). Also, according to the different methylation patterns two distinct clusters of tumors are categorized as driver mutations (Backman et al. 2017). Among 12 candidate CpG sites, one hypermethylated site (cg02119938) and one hypomethylated site (cg26870725) were suggested as important sites in PPGLs (Oishi et al. 2016). These

Table 1
The list of different molecules that had been shown as an indicator of malignancy in pheochromocytomas and paragangliomas.

Target Molecule	Detected Alteration	Detection Method	References
Cyclooxygenase-2	Overexpression	Immunohistochemistry Northern blot Western blot analyses	Salmenkivi et al. 2001
secretogranin II-derived peptide EM66	Immunoreactivity	Immunohistochemistry	Yon et al. 2003
hsp90	Overexpression	RT-PCR	Boltze et al. 2003; Giubellino et al. 2013
hTERT	Overexpression and Telomerase Activity	RT-PCR TRAP assay	Boltze et al. 2003; Salmenkivi et al. 2004; Scholz et al. 2007
nm23-H1	Down-regulation	qRT-PCR	Ohta et al. 2005; Saffar et al. 2011
TIMP-4	Down-regulation	qRT-PCR	Ohta et al. 2005
BRMS-1	Down-regulation	qRT-PCR	Ohta et al. 2005
TXNIP	Down-regulation	qRT-PCR	Ohta et al. 2005
CRSP-3	Down-regulation	qRT-PCR	Ohta et al. 2005
E-Cadherin	Down-regulation	qRT-PCR	Ohta et al. 2005
ERBB-2	Overexpression	Multiplex ligation-dependent probe amplification and immunohistochemistry	Yuan et al. 2008
c-erbB-2	Overexpression	Immunohistochemistry	Tavangar et al. 2010
Ki-67/MIB-1	Immunoreactivity	Immunohistochemical staining	Elder et al. 2003; Assadipour et al. 2017; Tavangar et al. 2010
PAM	Overexpression	Microarray analysis	Thouennon et al. 2007; Helman et al. 1989
QPCT	Overexpression	Microarray analysis	Thouennon et al. 2007; Helman et al. 1989
NPY	Overexpression	Microarray analysis	Thouennon et al. 2007; Helman et al. 1989
SDHB	Mutation		Amar et al. 2007
GATA3	Expression	Immunohistochemistry	Kouros-Mehr et al. 2006; Perrino et al. 2017
PTTG1	Expression	Immunohistochemistry	Amousha et al. 2015
MYCN	Mutation	Whole-exome sequencing	Wilzen et al. 2016
MYO5B	Mutation	Whole-exome sequencing	Wilzen et al. 2016
VCL	Mutation	Whole-exome sequencing	Wilzen et al. 2016
KMT2D	Overexpression	Whole-exome sequencing	Juhlin et al. 2015
galectin-3	Overexpression	Immunoreactivity	Saffar et al. 2011
FH	Mutation		Castro-Vega et al. 2014
ATRX	Mutation	Whole-exome sequencing	Fishbein et al. 2015

Abbreviations: RT-PCR – reverse transcriptase polymerase chain reaction analysis; qRT-PCR – quantitative real-time polymerase chain reaction; TRAP – telomeric repeat amplification protocol

sites were linked to *ACSBG1* (acyl-CoA synthetase bubble gum family member 1) and *MAST1* (microtubule-associated serine-threonine kinase 1), and there is a big possibility that epigenetic modifications in the malignant transformation of PPGLs is associated with *ACSBG1* silencing or *MAST1*overexpression (Oishi et al. 2016).

In the near future, a comprehensive molecular testing of PPGLs possibly will be used to decide therapeutic approaches and assess diagnosis and prognosis biomarkers. Considering the current development of next-generation sequencing-based genetic screening and liquid biopsy, this technology appears to be as a good option to improve personalized managements of both PPGLs molecular diagnosis and patient management (Burnichon et al. 2016; Khatami and Tavangar 2017).

Conclusions

In summary, genetic and epigenetic alterations that are differentially expressed in benign against malignant PPGLs were identified and reported. Some of them are a member of clustering genes like *SDHB,* while some others are different. Moreover, the reports indicate identification of approximately 25 differentially expressed genes and variations in methylated genes in malignant and benign tumors.

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