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Succinate dehydrogenase (SDH) deficiency, Carney triad and the epigenome

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ABSTRACT

In this report, we review the relationship between succinate dehydrogenase (SDH) deficiency and the epigenome, especially with regards to two clinical conditions. Carney triad (CT) is a very rare disease with synchronous or metachronous occurrence of at least three different tumor entities; gastric gastrointestinal stromal tumor (GIST), paraganglioma (PGL), and pulmonary chondroma. This condition affects mostly females and it is never inherited. Another disease that shares two of the tumor components of CT, namely GIST and PGL is the Carney-Stratakis syndrome (CSS) or dyad. CSS affects both genders during childhood and adolescence. We review herein the main clinical features and molecular mechanisms behind those two syndromes that share quite a bit of similarities, but one is non-hereditary (CT) whereas the other shows an autosomal-dominant, with incomplete penetrance, inheritance pattern (CSS). Both CT and CSS are caused by the deficiency of the succinate dehydrogenase (SDH) enzyme. The key difference between the two syndromes is the molecular mechanism that causes the SDH deficiency. Most cases of CT show down-regulation of SDH through site-specific hyper-methylation of the *SDHC* gene, whereas CSS cases carry inactivating germline mutations within one of the genes coding for the SDH subunits A, B, C, or D (*SDHA*, *SDHB*, *SDHC*, and *SDHD*). There is only partial overlap between the two conditions (there are a few patients with CT that have SDH subunit mutations) but both lead to increased methylation of the entire genome in the tumors associated with them. Other tumors (outside CT and CSS) that have SDH deficiency are associated with increased methylation of the entire genome, but only in CT there is site-specific methylation of the *SDHC* gene. These findings have implications for diagnostics and the treatment of patients with these, often metastatic tumors.

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Contents

1. Introduction

In this review, we report on recent studies of succinate

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dehydrogenase (SDH) deficiency and the latter's effect on the epigenome. This is particularly relevant for two genetic conditions, Carney triad (CT) and Carney-Stratakis syndrome (CSS), but also for "wild type" gastric stromal tumors (GISTs), paragangliomas, phaeochromocytomas and a few other tumors with SDH deficiency.

CT (OMIM# 604287) was originally described by Dr. J. Aidan Carney as the association of three uncommon neoplasms – gastric leiomyosarcoma or GIST, functioning extra-adrenal paraganglioma (PGL) and pulmonary chondroma (CHO)- in unrelated patients (Carney et al., 1977). CT is a very rare condition and the occurrence of those three tumors can be synchronous or metachronous (Haller et al., 2014). Young females (median 18 years old) are mostly affected from this disease, for reasons that remain unknown (Carney, 2009; Zhang et al., 2010). Although a decrease in the SDH enzymatic activity was detected in tumors from patients with CT, Matyakhina et al. (2007) could not identify any mutations in the genes coding for the four SDH subunits (*SDHA*, *SDHB*, *SDHC*, and *SDHD*, respectively, collectively known as the 'SDHx' genes) in a cohort of 37 patients with CT. However, this investigation also led to the description of a related disease, the dyad of PGL/pheochromocytoma (PHEO) and GIST, also known as CSS (OMIM# 606864) that affects both genders during childhood and adolescence. CSS is inherited as an autosomal-dominant trait and is never associated with the third component of the triad, CHO (Carney and Stratakis, 2002). Although there are some common features between the CSS and the CT, the relative frequency of the shared tumors in the two conditions is reversed: paraganglioma is predominant in CSS whereas GISTs are predominant as a manifestation in CT. It is important to mention that in most patients who develop GIST the sarcoma is symptomatic before paragangliomas or pheochromocytomas (Carney and Stratakis, 2002).

The SDH enzyme (also known as succinate-ubiquinone oxydoreductase) is a key enzyme in the mitochondrial citric acid cycle and electron transport chain. It is a highly conserved heterotetrameric protein that consists of two catalytic subunits that protrude into the mitochondrial matrix (SDHA and SDHB) anchored to the inner membrane by the other two subunits, SDHC and SDHD. The SDHC and SDHD subunits provide also the binding site for the ubiquinone (Fig. 1). The SDHx subunits are encoded by genes expressed in the nucleus. Following their import into the mitochondria, the SDHx subunits are further modified, folded and assembled into active forms. SDH, unlike most of the Krebs cycle enzymes, has no cytosolic analogue and comprises mitochondrial complex II, which is involved in the Krebs cycle and in electron transport chain (ETC). Complex II couples the oxidation of succinate to fumarate in the Krebs cycle with the electron transfer to the terminal acceptor ubiquinone in the ETC (Bardella et al., 2011; Scheffler, 1998).

2. SDH subunit mutations in paragangliomas and pheochromocytomas

Germline mutations in *SDHD*, *SDHB* and *SDHC* genes, were identified in patients with hereditary paragangliomas and pheochromocytomas (Astuti et al., 2001a; Baysal et al., 2000; Niemann and Muller, 2000) in the early 2000's (Astuti et al., 2001b; Gimm et al., 2000; van Nederveen et al., 2007). More recently, mutations in the genes encoding *SDHA* and *SDHAF2* were also linked to hereditary paragangliomas and pheochromocytomas (HPGL/PCC) (Burnichon et al., 2010; Hao et al., 2009). The genetic defects in the *SDH* genes predisposing to the HPGL/PCC syndromes are germline heterozygous inactivating mutations that typically affect the protein function. Loss of heterozygosity (LOH) in the tumor leads to complete loss of the enzymatic function of the SDH; therefore, the SDH subunit genes are classified as classical tumor suppressor genes (Gimenez-Roqueplo et al., 2001, 2002; Gimm et al., 2000).

3. Gastrointestinal stromal tumors (GISTS)

GISTs are the most common mesenchymal tumors of the gastrointestinal tract and they arise from the interstitial cells of Cajal (ICC) or from an ICC precursor (Rubin et al., 2005; Sommer et al., 2003). GISTs are divided into two categories based on the genetic defect they are harboring. The largest group includes GISTs that carry mutations in *KIT* (75%–80% of the cases) and *PDGFRA* (5%–15%) genes (Hirota et al., 1998; Huss et al., 2013; Rossi et al., 2015). The remaining ~10% of the gastrointestinal stromal tumors that are called "wild type" GISTs are classified into succinate dehydrogenase (SDH)-deficient and non-SDH-deficient groups; the latter group includes neurofibromatosis-1 (NF1)-mutant cases and others with mutations in the *BRAF*, *KRAS, PIK3CA* and in the *ETV6-NTRK3* fusion gene (Fig. 2) (Agaram et al., 2008; Brenca et al., 2016; Hostein et al., 2010; Lasota et al., 2016; Miettinen et al., 2006; Miranda et al., 2012; Patil and Rubin, 2015; Weldon et al., 2016).

3.1. Wild type (WT) GISTs without SDHx mutation in CT

Patients with CT usually present with symptoms of gastric "wild type" GISTs. These tumors are usually multiple with variable sizes (Weldon et al., 2016). They consist of mostly epithelioid tumor cells with pleomorphism commonly noted (Zhang et al., 2010). Immunohistochemical determination in this type of GISTs is positive for KIT expression (normal Cajal cells and the infiltrating mast cells) (Wada et al., 2016; Zhang et al., 2010). GISTs in CT are classified as low risk, however in a quarter of the patients a lymph node metastasis has been found after their first surgery (Wada et al., 2016).

Immunohistochemistry (IHC) for SDHB is helpful in making the diagnosis of an SDH-deficient GIST: loss of SDHB expression indicates that the tumor belongs to the non-KIT, non-PDGFRA-mutant or "wild type" category (Janeway et al., 2011; Killian et al., 2013). In SDH-deficient GISTs that do not harbor *SDHx* DNA defects, SDH deficiency results from an *SDHx* epigenetic down-regulation (Szarek et al., 2015) and leads to downstream activation of the HIF signaling pathway. Although, CT is mainly caused from SDH epimutation there are some rare cases where CT is associated with germline SDH defects (Boikos et al., 2016b).

Lasota et al. (2013) proposed that overexpression of type 1 insulin-like growth factor receptor (*IGF1R*) can be associated with SDH-deficient GISTs: the majority of SDH-deficient GISTs (89%) expressed high levels of IGF1R at the protein level. The exact molecular mechanism for *IGF1R* overexpression in these GISTs is currently unknown (Mahadevan et al., 2015). More recently, Killian et al. (2014) studied SDH deactivation through a genome-wide DNA methylation and expression study that included 59 SDH-deficient GISTs. They reported that 94% of their SDH-deficient GISTs that lacked actual SDH mutations showed *SDHC* promoter-specific CpG island hypermethylation and subsequent gene silencing. These results supported their hypothesis that *SDHC* epimutation could be the molecular mechanism that leads to succinate dehydrogenase enzyme dysfunction in most SDH-deficient GISTs that lack SDH mutations.

3.1.1. DNA methylation in CT

The promoter regions of the genes coding for *SDH* subunits harbor extensive CpG islands. We studied different types of tumors from patients with CT and CSS (Haller et al., 2014) and performed broad, high-resolution, and quantitative analysis of DNA methylation; we detected differential DNA methylation for the gene loci

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