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Dictating genomic destiny: Epigenetic regulation of pancreatic neuroendocrine tumours

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ABSTRACT

Pancreatic neuroendocrine tumours are a diverse group of neoplasms with an increasingly well-defined genomic basis. Despite this, much of what drives this disease is still unknown and epigenetic influences represent the next tier of gene, and hence disease modifiers that are of unquestionable importance. Moreover, they are of arguably more significance than the genes themselves given their malleable nature and potential to be exploited for not only diagnosis and prognosis, but also therapy. This review summarises what is known regarding the key epigenetic modifiers of disease through the domains of diagnosis, prognosis and treatment.

Contents

1. Introduction

Next generation sequencing and other new technologies have revealed an unparalleled insight into the genomic backbone of cancer. Many of the key epigenetic regulators of these genes remain poorly understood however. Epigenetics has been defined as the study of heritable changes in gene expression without modification of the underlying DNA sequence (Karpathakis et al., 2013). Epigenetic influences represent the next tier of gene, and hence disease modifiers that are of unquestionable importance. Moreover, they could be more significant than the genes themselves given their malleable nature and potential to be exploited for not only

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diagnosis and prognosis, but also therapy (Jones and Baylin, 2002; Jones and Martienssen, 2005).

Pancreatic neuroendocrine tumours (PNETs) are a heterogeneous group of tumours that generally convey a favourable prognosis and until recently, the genomic basis of disease had been relatively poorly understood. This has been improved somewhat by the recent works of Scarpa et al. who have provided some of the most insightful evidence yet regarding the genomic landscape of PNETs (Scarpa et al., 2017). Along with previously characterised mutations (eg. Multiple endocrine neoplasia 1; *MEN1* and von Hippel-Lindau; *VHL*), this group's analysis of 102 PNETs identified a surprising number of somatic mutations. Many of these genes (eg. mutY DNA glycosylase; *MUTYH* and Checkpoint kinase 2; *CHEK2*) control epigenetic processes, such as chromatin remodelling and DNA repair.

Whilst our understanding of the genomic drivers of disease is slowly progressing, there remains an additional paucity of information regarding the true epigenetic governors of PNETs (Karpathakis et al., 2013). This review summarises what is known regarding the key epigenetic modifiers of disease through the domains of diagnosis, prognosis and treatment.

2. Epigenetic regulation in cancer

There are numerous mechanisms by which epigenetic regulation of gene expression can be imposed in cancer (Jones and Baylin, 2002). The review of epigenetic regulators of neuroendocrine disease by Karpathakis and colleagues eloquently defines a timeline of significant epigenetic discoveries in cancer (Karpathakis et al., 2013). "Waddington's epigenetic landscape" (Waddington, 1957) in the mid-20th century was soon followed by the discovery of DNA methylation (Friedman et al., 1963) and then histone modification (Allfrey et al., 1964) in the 1960's. An emphasis of the role of gene methylation was the focus of research efforts leading into the early 21st century (Jones and Baylin, 2002), at which time the evolving role of micro-RNAs (miRNAs) and their importance in cancer was also discovered (Calin et al., 2004).

It is now recognised that epigenetic changes are a key event in cancer initiation and development (Jones and Baylin, 2002). Aberrant methylation changes for instance (hypo- and hypermethylation) have been widely demonstrated in numerous cancers, with the attendant gene silencing abnormalities and the downstream consequences of such changes (eg. chromosomal instability) being well defined at almost every stage in cancer progression (Jones and Baylin, 2002).

Such findings provide the principles upon which our epigenetic understanding of cancer are today based and revolve around three main mechanisms or constituents; DNA methylation, post translational histone modification and miRNAs. The remainder of this review involves a specific focus on epigenetic findings of special significance to PNETs within these three domains.

2.1. DNA methylation

2.1.1. Mechanism

DNA methylation occurs on the cytosine ring at the 5' position within CpG dinucleotides. Methylation at these sites is enacted by DNA methyltransferase enzymes (DNMTs), following which gene expression is altered (methylation induces gene silencing) and downstream signalling then influences the malignant process. To date, at least three families of DNMTs have been defined (DNMT1, 2 and 3) which are of varied significance within the context of malignancy and PNETs in particular (Okano and Xie SLi, 1998). Methylation can however be enacted by numerous other enzymes and pathways.

2.1.2. Findings in PNETs

Methylation related gene manipulation has been the most intensely investigated process within the realm of epigenetic PNET research. Much of this started in the late 1990's where functional PNETs (specifically gastrinomas) were the subject of some the first studies to examine gene methylation in a small cohort of 12 cases (Muscarella et al., 1998). Silencing of the p16/macrophage stimulating 1 gene (*p16/MST1)* was identified in up to 92% of tumours. More specifically, all tumours where homozygous deletion of the gene was not found, were shown to exhibit gene hypermethylation (ie. 58.3%); thus effectively leading to a comparative gene silencing effect with similar consequences. Subsequent studies on a larger cohort of 44 gastrinomas have also identified a high frequency of methylation changes (52%) in the p16/cyclin-dependent kinase inhibitor 4a (*p16/INK4a*) gene (Serrano et al., 2000). A lack of prognostic correlation between *p16/INK4a* methylation and outcome prompted this group to conclude only that methylation was likely an early event in tumour biology central to the pathogenesis of this disease.

The defining studies of House and colleagues were some of the first to identify large volume candidate gene promotor region methylation changes in PNETs (House et al., 2003). In a series of 48 PNETs, 87% were found to harbour aberrant hypermethylation in at least one of eleven candidate genes; the five most common being: Ras association domain-containing protein 1A (*RASSF1A*; 75%), *p16/INK4a* (40%), O-6-methylguanine-DNA methyltransferase (*O6-MGMT;* 40%), retinoic acid receptor beta (*RAR-beta*; 25%) and MutL homolog 1 (*hMLH1*; 23%). Beyond this, clinical significance was also demonstrated, with independent associations being defined between gene hypermethylation status and the outcomes of post resection early recurrence and reduced five year survival; particularly amongst patients who had node negative disease at diagnosis. These findings were most significant for those with tumours showing methylation at three or more loci of the candidate gene in question.

More specifically, the House et al. studies identified a high frequency of hypermethylation changes in the Ras-association domain gene family 1 (*RASSF1*); a finding that has been supported by subsequent studies (Dammann et al., 2003; Malpeli et al., 2011). *RASSF1* is a known tumour suppressor gene with a cell cycle arrest mechanism and has been implicated in numerous aspects of PNET disease through promoter region hypermethylation of numerous isoforms. Elevated expression levels of the *RASSF1A* and *C* isoforms in particular have been of particular note when comparing PNETs with normal control tissue (House et al., 2003; Malpeli et al., 2011).

In an effort to validate the functional impact of hypermethylation induced gene sequencing, *in vitro* studies have employed the use of 5-aza-2'-deoxycytidine (Habbe et al., 2007; Kim et al., 2013). This agent has been shown to reinstate tumour suppressor gene expression in association with suppressed cell proliferation (Habbe et al., 2007); thus reinforcing the pathological impact of methylation.

Gene promotor methylation is somewhat inconsistent across the different neuroendocrine tumours and even PNET subtypes, reflecting the heterogeneity of this disease (Chan et al., 2003; Wild et al., 2003; Liu et al., 2005; Choi et al., 2007; How-Kit et al., 2015). The early studies of Chan and co-workers were quick to identify vast differences in candidate gene methylation status between PNETs and carcinoid tumours (Chan et al., 2003). In their series of 11 PNETs and 16 carcinoid tumours, only methylation of the oestrogen receptor gene was more common in PNETs (vs carcinoids). These findings were subsequently supported by a larger series with a greater diversity of primary tumour sites (Liu et al., 2005). With regard to PNET subtypes, in one series, 44% of PNETs generally showed evidence of tissue inhibitor of metalloproteinase 3 (*TIMP3)*

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