EPIGENETIC ALTERATIONS IN ENDOCRINE-RELATED CANCER

("Epigenetics and endocrine cancer")

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Abstract

Aberrant epigenetics is a hallmark of cancer, and endocrine-related tumors are no exception. Recent research is identifying an ever-growing number of epigenetic alterations in both genomic DNA methylation and histone posttranslational modification in tumors of the endocrine system. Novel microarray and ultra deep sequencing technologies have allowed the identification of genome wide epigenetic patterns in some tumor types such as adrenocortical carcinoma, parathyroid and breast. However, in other cancer types, such as the multiple endocrine neoplasia syndromes and thyroid cancer, tumor information is limited to candidate genes alone. Future research should fill this gap and deepen our understanding of the functional role of these alterations in cancer, as well as defining their possible clinical uses.
Introduction

Epigenetics is defined as the study of those stable genetic modifications that result in changes in function and gene expression without altering the DNA sequence. The term was first described in 1942 by C.H. Waddington as the study of how genotypes give rise to phenotypes through programmed changes during development (Waddington 1942). Epigenetic mechanisms refer to changes in the interaction between DNA and histones which influences the degree of compaction of chromatin, allowing the genome to be differentially manifested depending on the stage of development, the type of tissue or the existence of disease. Among these mechanisms, DNA methylation and histone post-translational modifications coexist with histone variants, chromatin remodelers, small non-coding RNA molecules and polycomb and trithorax complexes. However, all such mechanisms in fact cooperate with each other, and also with other levels of regulation, to establish and maintain chromatin in either a condensate or non-condensate state, which ultimately determines gene expression profiles. In this review we will focus exclusively on DNA methylation and histone modifications.

DNA methylation is the most studied epigenetic mechanism to date and occurs in around 3% of cytosines, which precede guanines as part of the so-called cytosine-guanine dinucleotides (CpG) present in the genome (Hermann, et al. 2004). Recently non CpG methylation in stem cells has been described and it seems not to be directly associated with transcriptional repression, but rather to be associated with maintaining a pluripotent state (Lister, et al. 2009).

Some CpG sites in the genome are concentrated on so-called "CpG islands" located around 200 bp at several kb from the transcription start site of a gene (Antequera 2003). These CpG islands are present in 60% of genes and are usually non methylated, except in those genes subject to genomic imprinting, X chromosome inactivation or tissue
specific repression (Antequera 2003). In contrast, those CpG dinucleotides which are scattered throughout the genome are more methylated and located mainly in the bodies of genes, intergenic regions, repetitive sequences and transposons (Fedioiw, et al. 2012; Martin-Subero 2011). In the context of CpG islands, DNA methylation is sometimes associated with transcriptional repression, this being an important mechanism of gene regulation. DNA methylation can promote greater condensation of chromatin, preventing the access of transcription machinery. It has been proposed that, when CpG sites are part of non-coding, repetitive sequences and transposons, the role of methylation is to preserve chromosome stability by preventing the reactivation of mobile elements and maintain the integrity of chromosomes (Bird 2002). Through the maintenance of chromosomal stability and the regulation of gene expression, DNA methylation is crucial for processes such as cell differentiation and embryonic development (Fedioiw et al. 2012). It is carried out by a group of enzymes known as DNMTs (cytosine-5-DNA methyltransferases), which transfer the methyl group from S-adenosylmethionine (SAMe) to the C5 of cytosine. In mammals 5 DNMTs have been described, of which only three are active: DNMT1 is primarily involved in the maintenance of methylation patterns and has a preference for hemimethylated DNA which is frequently located at DNA replication sites during the cell cycle (S phase) (Qin, et al. 2011). DNMT3A and DNMT3B are known as the "de novo DNMTs," since they seem to possess the ability to establish profiles of DNA methylation, and do not discriminate between hemimethylated and unmethylated DNA (Chedin 2011) although further studies have demonstrated that both types of enzyme play a role in the maintenance of methylation as well as in methylation-dependent repression of specific oncogenes in cancer cells (Fernandez, et al. 2012). These enzymes determine the overall methylation profiles in the early stages of embryonic development, in germ cells and
cooperate in the establishment and maintenance of DNA methylation profiles (Fatemi, et al. 2002).

Histones have an amino terminal tail consisting of 20-35 amino acid residues, with a highly conserved sequence that is susceptible to modifications such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation and ADP-ribosylation. These are, in the majority, dynamic and reversible changes. The enormous variety of potential modifications and their possible combinations generate a wide range of functional responses known as the "histone code". Histones play a role in the establishment of structural domains of chromatin and the regulation of DNA functions such as transcription, repair, replication and condensation of chromosomes. Two of the best studied modifications are the acetylation and methylation of histones. The former is carried out by HATs (histone acetyltransferases) and reversed by HDACs (histone deacetylases) while the latter is thought to take place at the lysine and arginine residues, and can incorporate one to three methyl groups for each residue (Zhang, et al. 2012). At the transcriptional level, the effect of methylation varies as a function of its extent and the specific residue affected and it is also involved in establishing chromatin structural domains. The enzymes responsible for this modification are known as HMTs (histone methyltransferases), are substrate specific and employ SAMe as a donor of methyl groups, while the reverse process is carried out by HDMs (histone demethylases) (Li, et al. 2012).

**Epigenetic alterations in Tumors of the Endocrine System**

In the last 20 years, advances in the field of endocrine oncology have enabled the genetic basis of some hereditary endocrine tumors to be uncovered, and they have also contributed to increasing knowledge of certain sporadic diseases, and consequently the
development of new diagnosis or treatment methods. In addition, the contribution of epigenetic mechanisms in tumor development has been widely described. In this review we will focus on those endocrine tumors where the role of certain epigenetic mechanisms (DNA methylation and histone modifications) has been demonstrated. Endocrine tumors affect parts of the body that secrete hormones and include: adrenal gland tumor (adrenocortical carcinoma), islet cell tumors (gastrinoma, vipoma, glucagonoma, somatostatinoma), neuroendocrine tumors (such as pheochromocytoma), parathyroid and thyroid carcinomas, among others. In the following sections, for the purpose of discussion, these endocrine tumors will be divided into those which are hereditary (MEN syndromes) and those which are sporadic (thyroid, parathyroid, breast and ovarian, prostate, pheochromocytoma, paraganglioma, adrenocortical and lung neuroendocrine tumors).

1. Hereditary Endocrine Tumors

1. The Multiple Endocrine Neoplasia (MEN) Syndromes

Multiple endocrine neoplasia (MEN) syndromes predispose people to develop endocrine tumors. The major glands affected by the MEN syndromes are the pituitary, thyroid, parathyroid, adrenal and pancreas. There are two different MEN syndromes, MEN1 and MEN2, which are similar although there are important differences.

Multiple Endocrine Neoplasm Type 1 (MEN1) syndromes (prevalence of 3 per 10000) are characterized by the development of tumors of the parathyroid and, pituitary glands and the pancreas (Thakker, et al. 2012). The genetic mutation responsible for MEN1 spans 9.8 kb of chromosome 11q13 (Chandrasekharappa, et al. 1997). Germ-line mutations in MEN1 have been found in the vast majority of MEN1 kindreds, and somatic MEN1 mutations have also been reported.
in sporadic parathyroid adenomas, pituitary and lung tumors, insulinomas and gastrinomas (Debelenko, et al. 2000; Delemer 2012; Hasani-Ranjbar, et al. 2011). The protein product of \textit{MEN1}, termed menin, is a tumor suppressor protein ubiquitously expressed in the nucleus. It is reported to interact with transcription factors such as JunD, Smad3, but has also been found as a component of a multiple protein complex (through interaction with the trithorax group proteins MLL2, \textit{mixed lineage leukemia}, and MLL) which displays a histone H3 lysine 4 methyltransferase activity. Trimethylated H3K4 is an epigenetic mark typically associated with transcriptionally active chromatin, and menin may function as a tumor suppressor by regulating histone methylation in promoters of specific target genes that govern neuroendocrine cell growth and differentiation, in a similar way to described in leukemogenesis (Chang, et al. 2011; Murai, et al. 2011; Thiel, et al. 2012) (Table 1)

Insulinomas are extremely rare but are the most common neuroendocrine tumor associated with MEN1 in the pancreas: seldom malignant they are derived from beta cells with an uncontrolled secretion of insulin that results in hypoglycemia (Mathur, et al. 2009). The work developed by Karnik et al. (Karnik, et al. 2005) demonstrated that in pancreatic endocrine cells menin interacts with \textit{p27} and \textit{p18} promoters; two cyclin-dependent kinase inhibitors that play an important role in islet growth control. Menin, through MLL2 interaction, regulates the histone methyltransferase activity of this TrxG complex member and, as a transcriptional coactivator, promotes histone methylation of \textit{p27} and \textit{p18}. Men1 inactivation disrupts \textit{p27} and \textit{p18} expression and alters islet growth control and tumor suppression. Moreover, Fontaniére et al. have demonstrated in a mice model that \textit{Men1} disruption was not enough to trigger tumorigenesis of \textit{β}-cells and that the gene expression profile of insulin-like growth factor (\textit{IGF}) was also deregulated. They showed that \textit{IGF2} was overexpressed in \textit{Men1} mutant mice, as happens in other
abnormal B cell proliferations (Vasavada, et al. 2006) as well as in insulinomas (Hoog, et al. 2001), and that this overexpression is a consequence of the hypermethylation of the intragenic differentially methylated regulatory regions (DMR2) of the Igf2 gene, which increases the level of transcription through this epigenetic mechanism.

Pituitary tumors occur in 54 to 80% of patients with MEN-1, and prolactinoma is the most common (41-76%). Yoshino et al. showed promoter hypermethylation in at least one of the cell cycle regulator genes (RB1, p14ARF, p15 (INK4b), p16, p21, p27 and p73) in pituitary tumors, (Yoshino, et al. 2007). Interestingly, genes of the RB1 pathway were methylated in 85% of the samples, which suggests that, in addition to MEN1 mutations, methylation could be a molecular alteration that contributes to these tumors. These results were also confirmed by Bello et al. (Bello, et al. 2006), who found promoter hypermethylation in the RB1, p14 (ARF), p16, p73 genes, as well as the TIMP3 (Metalloproteinase inhibitor 3), MGMT (-6-methylguanine-DNA methyltransferase), DAPK, THBS1 and caspase-8 genes, some of which are involved in the apoptosis processes (DAPK and caspase-8), DNA repair (MGMT) or have anti-angiogenic properties (THBS1). Additionally, reduced expression of the fibroblast growth factor receptor (FGFR2), a member of the FGF family with a critical role in pituitary development, in human pituitary tumors has been associated with gene promoter methylation (Zhu, et al. 2007).

The Multiple Endocrine Neoplasia type 2 (MEN2) is characterized by a very high risk (95%) of developing medullary thyroid cancer (MTC). It is divided into three clinical subtypes: MEN2a, MEN2b and FMTC (familial medullary thyroid carcinoma). MEN2a, characterized by the presence of MTC at the beginning of adulthood (50%), pheochromocytoma and parathyroid hyperplasia (no adenomas) (20-30%). Parathyroid hyperplasia does not however develop in MEN2b, which is characterized by MTC in
early childhood and pheochromocytomas (often 50%). FMTC affects several members of the same family, where pheochromocytoma and hyperparathyroidism are not present. MTC originates from C cells, derived from the neural crest, secrete calcitonin (CTN) and glycoprotein carcinoembryonic antigen (CEA). Mutations in the RET protooncogene, which encodes a tyrosine kinase receptor, have been described as the main cause of MEN2a, MEN2b and FMTC. Moreover, promoter methylation of RASSF1A could also be involved in thyroid cancer development due to its high incidence in MTC (85%) (Schagdarsurengin, et al. 2002). RASSF1 encodes a signaling protein (tumor suppressor) that functions through a pathway involving Ras, a component of the phosphatidylinositol 3 kinase (PI3K)/Akt pathway, and is mainly inactivated by methylation (Brait, et al. 2012). This phenomenon is more frequent in those tumors that develop distant metastasis and is also found in the more aggressive forms of MTC (table 1).

2. Sporadic Endocrine Diseases

1. Thyroid Tumors

The thyroid tumors can be classified by their degree of clinical aggressiveness: the least aggressive forms include papillary thyroid carcinomas (PTCs), the most frequent (50-90%), which can metastasize early in the cervical lymph nodes, and follicular thyroid carcinomas (FTCs), which spread primarily by hematogenous dissemination; the medullary thyroid carcinoma (MTC), which causes early metastasis (lung, bone) originating both via the lymph and blood; and the most aggressive, anaplastic thyroid carcinoma (ATC) with early distant metastasis. Gene promoter methylation of tumor suppressor genes such as E-cadherin, PTEN, RASSF1A and FGFR2 has been described as part of the pathology of the thyroid tumors (table 1). The PTEN gene encodes a
phosphatase that blocks the signaling of the PI3K/Akt pathway which is constitutively
activated in FTC. The RASSF1A, RASSF2 and RASSF10 genes (Ras association domain
family signaling proteins) are methylated in PTC, FTC, and ATC. Ras is a component
of the PI3K/Akt pathway, suggesting a relationship between PTEN and Ras proteins in
the development of this pathology (Brzezianska and Pastuszak-Lewandoska 2011;

Other tumor suppressor genes TIMP3, SLC5A8, and DAPK are also aberrantly regulated
by methylation in thyroid tumors (Brait et al. 2012; Porra, et al. 2005). TIMP3 is a
tissue inhibitor of metalloproteinases with important roles in inhibiting angiogenesis,
invasion and metastasis, which limits the spread of tumoral cells (Anania, et al. 2011).
SLC5A8, a member of the sodium solute symporter family (SLC5), and DAPK, a
calcium/calmodulin-dependent serine threonine kinase, both exert proapoptotic activity,
which suggests the possibility that their inactivation could block apoptotic processes
and thus facilitate invasion of the tumor cells in PTC. These results reveal the
importance of DNA methylation in the regulation of this gene’s expression and point to
its possible functional role in thyroid cancer.

Recently our group has developed genome methylation profiling of thyroid cancer,
allowing the description of differential DNA methylation patterns for the differentiated
and the non-differentiated subtypes. These latter are characterized by aberrant promoter
hypomethylation, which could be used in diagnosis and/or prognosis in this type of
cancer (Rodriguez-Rodero, et al. 2013)

2. Parathyroid Tumors

Parathyroid tumors are an abnormal growth in the parathyroid gland, and usually cause
hyperparathyroidism. Most are benign adenomas (85 %), and carcinomas are very rare
(0.5-1%). Overexpression of cyclin D, and deletions of the retinoblastoma gene or
BCRA2 genes on chromosome 13, have all been identified as possible causes of these tumors (Mallya, et al. 2010; Shattuck, et al. 2003).

Promoter hypermethylation of the tumor suppressor gene hypermethylated in cancer 1 (HIC1), has been shown to be frequently repressed in parathyroid cancer (Svedlund, et al. 2012). A growth-regulatory role has been assigned to HIC1 in the parathyroid glands and it is suggested that its down-modulation might be an early event in tumoral transformation, where, in addition to DNA methylation, other epigenetic mechanisms such as repressive histone marks (H3K27me2/3) play a part (table 1).

Similar to in thyroid tumors, promoter hypermethylation in RASSSF1 and APC genes has also been described in parathyroid carcinomas (Juhlin, et al. 2010) (Sulaiman, et al. 2013). A differential methylation pattern compared with normal parathyroid tissue was observed in parathyroid adenomas (367 genes were significantly altered) and parathyroid carcinomas (175 genes) (Starker, et al. 2011). In addition, CDKN2B, p16, WT1, SFRP1, SFRP2 and SFRP4 were hypermethylated in parathyroid carcinomas and showed reduced expression, which was reverted by 5-aza-2'-deoxycytidine, a demethylating agent, demonstrating the importance of alterations in cell cycle regulation in parathyroid tumors (Starker et al. 2011).

3. Breast Cancer

There are two main types of breast cancer: ductal carcinoma, which is the most common form, and lobular carcinoma. Breast cancer is a heterogeneous disease caused by interactions between inherited and environmental risk factors that lead to the progressive accumulation of genetic and epigenetic changes in breast cells, where a family history of breast cancer is the strongest risk factor for the disease (20%).

The role of epigenetic processes in breast cancer has been widely reported (Bediaga, et al. 2010; Huang, et al. 2011) (table 1). Methylation of the BRCA1 gene promoter has
been described in sporadic breast tumors, and suggested as a prognosis factor since it is more frequent in the invasive forms of breast cancer (Bosviel, et al. 2012). A relationship between $BRCA1$ hypermethylation and tumor stage was also described in ovarian cancer, the gene being hypermethylated in stages II and III when compared with stage I, which supports the idea that loss of $BRCA1$ expression is correlated with a more advanced stage of ovarian cancer (Wang, et al. 2013), and suggests the potential of this gene as a biomarker for ovarian and breast cancer.

Many breast cancers are sensitive to the hormone estrogen, which can contribute to tumor development. Those cancers with estrogen receptors on the surface of the cancer cells are termed estrogen receptor positive (ER positive). The estradiol in the mammary gland comes from ovarian synthesis, external glandular tissues (i.e. fat deposits) and the mammary gland itself. This hormone exerts mitogenic effects on breast cells leading to neoplastic transformation by increasing the rate of cell proliferation (Berstein, et al. 2010). The activities of estrogens are mediated by the two intracellular estrogen receptors (ERs), ER$\alpha$ and ER$\beta$, which are encoded by the genes $ESR1$ and $ESR2$ respectively and function as transcription factors to regulate gene expression. ER$\alpha$ is expressed in the great majority of breast tumors (75%) (Er$\alpha$- positive) and is frequently associated with a better prognosis and responsiveness to hormone treatment, while ER$\alpha$ negative breast tumors are associated with poorer prognosis and greater malignancy (Xie, et al. 2012). One mechanism involved in suppressing ER$\alpha$ expression in ER-negative tumors is aberrant methylation of CpG islands at the ER$\alpha$ promoter, which is present more frequently in metastatic tumors (associated with adverse clinical outcome) and may represent a key mechanism to hormone resistance (Nass, et al. 2000). This negative prognosis of ER negative tumors could also be mediated by the downregulation of the $MTA1$ (Metastasis tumor antigen 1) gene (observed when breast
cancer invasive cell lines are treated with demethylating agents) which permits the expression of Era (Mao, et al. 2012). The role of this protein in tumor aggressiveness has been confirmed by its role in inducing the pulmonary metastasis in breast cancer (Pakala, et al. 2013). Moreover, ERs work in conjunction with HATs and JMJD2B, a histone lysine-specific demethylase. Distal to the ER binding site there is an enrichment of H3K9me3 that acts as a repressive mark of transcription. Upon E2 (17 B estradiol) activation, JMJD2B expression is induced, it is recruited to ERa target sites and demethylates H3K9me3. Moreover, this interaction with the receptor also allows the recruitment of histone modifying enzymes, like HATs, to facilitate the transcription of ER responsive genes such as Myb, Myc or CCND1 (which alter cell cycle progression and may contribute to tumorigenesis), which could increase breast cell division and promote tumor growth (Shi, et al. 2011).

Other tumor suppressor genes such as p16 and RASSF1A or SLC25A43 (Solute carrier family 25A member 43) have also been found hypermethylated in breast cancer (Lindqvist, et al. 2012; Wang, et al. 2012; Xu, et al. 2012); RASSF1A in particular could be used as a prognostic marker in breast cancer (Lindqvist et al. 2012; Wang et al. 2012; Xu et al. 2012), and its role in cancer has also been described (see earlier sections) in non small cell lung and thyroid tumors. Recently, Faryna et al. demonstrated higher methylation in BCAN, HOXD1, KCTD8, KLF11, NXPH1, POU4F1, SIM1, and TCF7L1 genes in low-grade breast tumors (Faryna, et al. 2012). These results were confirmed by van Hoese et al. (van Hoesel, et al. 2013) who demonstrated an increase in promoter methylation in Methylated-IN-Tumour (MINT)17, MINT31, RARβ2 and RASSF1A genes, associated with a poor prognostic of the disease.

4. Ovarian Cancer
Stromal tumors (frequency < 3%) arise from structural cells that hold the ovary together and produce the female hormone estrogen. They often produce estrogen and inhibin A and B. Less often, they produce androgens (male hormones). Dhillon et al. found promoter methylation in a group of genes: FHIT (28%), a tumor suppressor gene also hypermethylated in other tumor subtypes; FNACF (24%), which interacts with BRCA1 and BRCA2 pathways; Cyclin D2 (12%), involved in the regulation of transition from G1 to S during the cell cycle and seemingly exerts a function as tumor suppressor gene in this type of cancer; BCRA2 (4%), which may play a role in regulation of the cell cycle during proliferation and differentiation; and RUNX3 (56%), which belongs to the RUNT domain genes and also has a tumor suppressor role (Dhillon, et al. 2004b) (table1).

Dhillon et al. also showed promoter hypermethylation in p16, BRCA1, RASSF1A, ER-α, TMS1, TIMP3, Twist, GSTP1, AR, and hMLH1 genes in ovarian cancer (Dhillon, et al. 2004a). TIMP-3, which is thought to suppress primary tumor growth, is also downregulated in prostate cancer by methylation and histone methylation (Shinojima, et al. 2012). In sporadic breast tumors, p16, MGMT, VHL, MLH1 and BRCA1, have also been shown to be important as prognostic factors (Bosviel et al. 2012), and all are DNA repair and tumor suppressor genes that have been demonstrated to be epigenetically inactivated by DNA methylation in cancers. RASSF1A hypermethylation is associated with a shorter recurrence time, in ovarian, or thyroid tumors (Brzezianska and Pastuszak-Lewandoska 2011; Buckingham, et al. 2010; Schagdarsurengin et al. 2010; Schagdarsurengin et al. 2009). p16 silencing by this epigenetic mechanism, seems to be an early event in the development and progression in ovarian cancers (Dhillon et al. 2004a).

5. Prostate Cancer
This is the second most frequent tumor in men (accounting for 11.7% of all male tumors). Prognosis improves with early detection (PSA antigen), and an estimated 58% of tumors are diagnosed at an early stage. Among the etiological causes of the disease are environmental and dietary factors (high-fat diet, excess weight), hormonal factors (androgens play an important role in the initiation and promotion of prostate cancer) and genetic factors such as the \textit{BRCA2} gene or \textit{ELAC2} (individuals with affected relatives respectively have a risk of 3.1 and 4.3 times higher compared with healthy controls) (Wallner, et al. 2011). The majority of prostate tumors are adenocarcinomas, while less than 1% are neuroendocrine tumors (Cohen 2005). Valentini et al, demonstrated that when human prostate androgen-dependent cancer cell line LNCaP was treated with valproic acid (VPA) an inhibitor of HDAC, this component induced neuroendocrine-like differentiation in this cell line as well as a down-regulation of androgen receptor protein and a reduction in prostate-specific antigen (Valentini, et al. 2007). Moreover, prostate cancer cells commonly exhibit promoter hypermethylation, which causes gene repression in the acquisition and maintenance of the neoplastic phenotype. \textit{GSTP1} is the most frequently methylated gene in prostate cancer. Glutathione S transferases (GST) comprise a family of enzymes involved in the detoxification of xenobiotics and oxygen radicals. Absent or diminished expression of the \textit{GSTP1} gene in prostate cancer has been reported, and is commonly due to CpG island \textit{GSTP1} promoter region methylation in the majority of prostate tumors (>90%), a phenomenon that is rarely detected in normal prostate or benign prostatic hyperplasia (BPH) tissues. This gene has also been described as hypermethylated in breast carcinoma, an early event, which suggests its utility as a biomarker (Fukushige and Horii 2013; Mahapatra, et al. 2012; Saxena, et al. 2012; Song, et al. 2013). The DNA alkyl-repair gene O6-methylguanine DNA methyltransferase (MGMT), which removes mutagenic and cytotoxic alkyl adducts
from genomic DNA, has also been found hypermethylated in this type of tumor (Kang, et al. 2004) (table 1).

The prostate responds to sex hormones through specific receptors. The male hormones (Testosterone and 5-dihydrotestosterone) exert their actions mediated by the androgen receptor (AR). Epigenetic changes, including CpG methylation and histone acetylation, play important roles in the regulation of AR pathway signaling, but the frequency of AR methylation is low in prostate cancer (Nakayama, et al. 2000). Estrogens also exert their effect on the prostate through the estrogen receptors ER1 and ER2, which are frequently methylated in prostate cancer and correlated with tumor progression (Li, et al. 2000). Hypermethylation events in prostate cancer also include genes involved in cell cycle regulation, like the tumor-suppressor gene p16, a cyclin-dependent kinase inhibitor (Verdoodt, et al. 2011) or RASSFLA (found in 49-99% of tumors), mentioned in earlier sections, which are also associated with other tumor types, and whose deregulation can cause DNA repair failures and Ras-dependent growth control in cancer cells, and which are associated with the most aggressive forms of prostate cancer (Amin and Banerjee 2012). Another hypermethylated tumor suppressor gene in prostate cancer is APC (adenomous polyposis coli), associated with poor prognosis (Delgado-Cruzata, et al. 2012).

Invasion and metastasis are also present in prostate cancer, and some of the genes involved in this process (CDH1, CD4 and TIMP-3) are also regulated by promoter methylation: E-Cadherin (CDH1), an important member of the cadherin family of cell adhesion molecules, is strongly reduced by promoter methylation in human prostate tumors and disruption of the cell adhesion system can lead to tumor infiltration and metastasis (Li, et al. 2001); CD44, which encodes for a protein involved in matrix adhesion (Lou, et al. 1999); and tissue inhibitors of metalloproteinases (TIMP-3), whose
promoter region was found to be methylated in 97% of prostate tumors, allowing MMP
expression, tumor growth, invasion, and tumor-induced angiogenesis (Jeronimo, et al.
2004).

Hypomethylation is another phenomenon frequently observed in a wide variety of
malignancies including prostate cancer (Bedford and van Helden 1987) and could
contribute to tumoral transformation through multiple oncogene activation (i.e. MYC and
H-RAS) and by chromosome instability.

Among the posttranslational modifications in histones, methylation of arginine and
lysine can be associated with either gene activation or repression. Methylation of lysine
9 in histone 3 (H3K9) is linked to repression of AR target genes, which may include
tumor suppressor genes (GAS2, PIK3CG and ADRB2), and histone H3K4 methylation is
associated with AR gene activation. H3K4me1 and H3K4me2 are selectively enriched
at the AR enhancers of UBE2C and CDK1 genes (M-phase cell cycle genes) or
oncogenes, facilitating AR upregulation of these genes to promote growth, suggesting a
role in prostate tumorigenesis. Moreover, increased H3K4me3 in prostate cancer cells
correlates with the activation of genes involved in cell growth and survival (i.e.
FGFR1 and BCL2) which seem to be responsible for poor clinical outcome in prostate

EZH2 (Enhancer of zeste homolog 2) has been found overexpressed in prostate cancer,
with higher expression in those which are metastatic. This protein is a member of the
Polycomb-repressive complex 2 (PRC2), which causes trimethylation of histone H3 on
Lys 27 (H3K27) and gene repression. Overexpression of EZH2 seems to be involved in
progression and invasion of tumoral cells, through the silencing of tumor repressor
genes like ADRB2, CDH1, PSP94, and DAB2IP (Ren, et al. 2012). Similarly, Babbio et
al. demonstrated that UHRF1 protein, frequently overexpressed in prostate cancer and
other tumors, might play a link role between different epigenetic mechanisms, and that it is correlated with poorer prognosis in prostate cancer patients (Babbio, et al. 2012). This protein binds specifically to methylated H3K9 and promotes DNA condensation and gene suppression, and is a strong candidate for a significant role in malignant transformation and tumor progression because its expression is related to downregulation of the tumor suppressor genes ACPP, CBX7 and GAS1 as well as it having a parallel expression to that of EZH2, which would indicate similarity of their roles in tumor progression.

6. Pancreatic Cancer

Pancreatic neoplasms arise from the endocrine and exocrine portions of the organ, where the most frequent form are the ductal adenocarcinomas (exocrine). The pancreatic neoplasms that develop from the endocrine portion include gastrinoma, glucagonoma, vipoma, somatostatinoma and insulinoma (see earlier section). The tumor suppressor gene p16, a cell cycle regulator, is frequently methylated in other tumors such as ovarian (Abou-Zeid, et al. 2011; Bammidi, et al. 2012) and this promoter methylation was also observed in 52% of gastrinomas in a study by Serrano et al. (Serrano, et al. 2000) as an early event, independent of the stage or localization of the disease. However, only limited studies have been conducted in relation to the role of epigenetic mechanisms in other endocrine pancreatic tumors (table 1).

7. Adrenocortical Carcinomas

Adrenocortical cancer is rare (0.5 to 2 cases per million) though very aggressive. The majority of tumors are functional (60% of patients) (Lafemina and Brennan 2012). Nonfunctional tumors (40%) present as incidentalomas, frequently affect older people and have poorer prognosis, they do not secrete any hormones or present specific symptoms. (Lehmann and Wrzesinski 2012).
The first genome wide DNA methylation profiling in adrenocortical tumors (ACT) showed that malignant tumors have global hypomethylation compared with normal tissue or benign tumors (Rechache, et al. 2012). These results are similar to those described in our group with the aggressive forms of thyroid tumors when compared with less aggressive subtypes and normal thyroid tissues (Rodriguez-Rodero et al. 2013). Rechache et al. showed that differences in methylation profile were higher between normal and primary malignant and metastatic samples than between normal and benign tumors. Moreover, 52 hypermethylated and down-regulated genes in adrenocortical carcinomas (ACC) were also identified (Rechache et al. 2012). Similarly, Fonseca et al. conducted genome wide methylation studies, finding 212 CpG islands in promoter regions that were significantly hypermethylated and which might contribute to pathology development (Fonseca, et al. 2012). Barreau et al. focused their analysis on promoter CpG islands of exclusively tumoral samples (84 adrenocortical adenoma and 51 adrenocortical carcinoma) and found that adrenocortical carcinomas were more hypermethylated than adenomas, in accordance with previous results (Barreau, et al. 2013). They also described that adrenocortical carcinomas could be subdivided into samples which are slightly more methylated (non-CIMP) and those that are highly methylated (CIMP). Finally, Suh et al. have demonstrated that treatment of adrenocortical tumors with Decitabine (5-aza-2'-deoxycytidine) reverses DNA promoter methylation of those genes with an antitumoral function (Suh, et al. 2010) (table 1).

8. Pheochromocytomas and Paraganglioma

Pheochromocytoma usually arises from the adrenal medulla. It is a catecholamine (adrenaline and noradrenaline) producing tumor arising from chromaffin cells derived from the sympathetic nervous system. Pheochromocytomas can also develop extra-adrenally (from chromafin cells), when they are called Paragangliomas. Both tumor
types mostly occur sporadically, although the hereditary syndromes multiple endocrine neoplasia (MEN) 2a and 2b, von Hippel Lindau disease (VHL), neurofibromatosis type 1 (NF1) and paraganglioma syndromes (PGL) 1, 2 and 3, are all associated with the development of pheochromocytoma/paraganglioma (see MEN2 section). Mutations in the proto-oncogene RET or the tumor suppressor genes VHL, NF1, SDHD, SDHC, and SDHB predisposes to tumor development in these disorders. Recently, Sandgren et al. found an increase in EZH2 expression in tumor samples when compared to normal adrenal medulla (Sandgren, et al. 2010). The up-regulation of this polycomb-associated methyltransferase, which specifically methylates H3K27, may be of some significance in pheochromocytoma tumorigenesis as it may contribute to silencing tumor suppressor genes (Sandgren et al. 2010). Furthermore, EZH2 overexpression has also been observed in other cancers types (Deb, et al. 2013).

In paragangliomas, p16 is commonly downregulated and may contribute to tumor development. Hypermethylation of p16 is associated with a poor prognosis and seems to contribute to a reduction in the amount of the protein, as has also been observed in follicular lymphoma and laryngeal squamous cell carcinoma (LSCC) (Krajnovic, et al. 2013; Pierini, et al. 2013). However, this hypermethylation was not observed in MEN2/RET associated paragangliomas (Kiss, et al. 2008; Kiss, et al. 2013; Muscarella, et al. 2008).

9. Lung Neuroendocrine Tumors

In small cell lung cancer (SCLC), derived from pulmonary neuroendocrine cells, genome-scale analysis of methylation changes developed in primary SCLC and SCLC cell lines by Kalari et al. found a group of 73 genes to be aberrantly methylathed in more than 77% of primary SCLC tumors (Kalari, et al. 2012). Most of these genes were transcription factors or involved in processes of neuronal differentiation (NEUROD1,
HAND1, ZNF423 and REST). The authors have hypothesized that inactivation of these transcription factors and proteins may cause a differentiation defect of neuroendocrine cells. Similarly, HAND1 methylation is closely associated with poor survival in patients with gastric cancer, which raises the possibility of using this gene as a potential biomarker for diagnosis or prognostic evaluation (Shi, et al. 2012). In addition, other epigenetic alterations, such as loss of histone H4 acetylation at lysine16 (H4K16ac) and trimethylation at lysine 20 (H4K20me3), has also been described in this type of tumor (Li, et al. 2011).

Conclusion

The biological relevance of epigenetic changes in pathological situations such as cancer has been widely documented, facilitating the possibility of applying the specific findings in relation to alterations as biomarkers in various aspects of patient care. In recent years, advances in endocrine oncology have greatly improved the molecular tools available for increasing the understanding of the mechanisms involved in the development of such diseases. The field of epigenetics undoubtedly plays a role in these pathologies. Genome wide methylation arrays has revealed the presence of certain genes with a tumor suppressor role where the promoter is aberrantly methylated, which may contribute reduced control of angiogenesis and thus facilitate tumor spread. In this review we have focused our attention on two primary epigenetic changes, DNA methylation and histone modifications, both of which play an important role in gene expression and chromatin structure. The reversible nature of epigenetic modifications allows the possibility of interventions to reverse such changes in order to treat the disease or halt its development.

Although there are many types of endocrine tumors, and for some only a small number of candidate genes have been analyzed, in this review we have described some common
epigenetic alterations to both hereditary and sporadic endocrine tumors (table 1). For
example, the methylation of the RASSF1A gene, related to several apoptotic and cell
cycle checkpoint pathways, or the hypermethylation of p16; although in both cases the
same alterations have also been found in tumor types other than endocrine. Further
epigenomic studies at the genome-wide level will be needed to identify any possible
specific epigenetic alterations in endocrine tumors with respect to other tumor types,
and whether these changes affect specific pathways.

The epigenetic mechanisms we have described contribute substantially, not only to
understanding the progress of the pathology, but also to the search for diagnostic or
prognostic markers and in the development of epigenetic drugs with possible
applications in the treatment of these tumors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as
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Table 1. Epigenetic alterations in endocrine tumors.

<table>
<thead>
<tr>
<th>ENDOCRINE NEOPLASIA</th>
<th>EPIGENETIC ALTERATION</th>
<th>REFERENCES</th>
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<tbody>
<tr>
<td><strong>Hereditary endocrine tumors</strong></td>
<td></td>
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<tr>
<td><strong>MULTIPLE ENDOCRINE NEOPLASIA 1 (MEN1)</strong></td>
<td>Menin interaction with the trithorax proteins MLL2 and MLL, with histone H3 lysine H4 methyltransferase activity.</td>
<td>Murai, M.J. et al. 2011</td>
</tr>
<tr>
<td><strong>MULTIPLE ENDOCRINE NEOPLASIA 2 (MEN2)</strong></td>
<td>Aberrant promoter methylation in RASSF1A</td>
<td>Schagdarsurengin et al. 2002</td>
</tr>
<tr>
<td><strong>Sporadic endocrine tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PARATHYROID TUMORS</strong></td>
<td>Aberrant promoter methylation in H1C1 and RASSSF1</td>
<td>Svedlun, et al. 2012 Juhlin et al.2010</td>
</tr>
<tr>
<td><strong>OVARIAN CANCER</strong></td>
<td>Aberrant promoter methylation BRCA1,2, FHIT, FNACF and p16</td>
<td>Dhillon et al, 2004</td>
</tr>
<tr>
<td><strong>PROSTATE CANCER</strong></td>
<td>Aberrant promoter methylation of GSTP1, MGMT, p16, CDH1 and RASSF1A</td>
<td>Kang et al, 2004 Mahapatra et al, 2012 Verdoot et al. 2011</td>
</tr>
</tbody>
</table>

*MLL2*: mixed lineage leukemia; *RASSF1*: Ras-association domain family; *PTEN*: phosphatase and tensin homolog; *TIMP3*: TIMP metallopeptidase inhibitor; *SLC5A8*: solute carrier family; *DAPK*: Death-associated protein kinase; *H1C1*: breast cancer 1; *FHIT*: fragile histidine triad protein; *FNACF*: Fanconi Anemia gene F; *GSTP1*: glutathione S-transferase P1; *MGMT*: O-6-methylguanine-DNA methyltransferase. *CDH1*: E-cadherin gene; *SPARC*: secreted protein, acidic, cysteine-rich (osteonectin); *TFP12*: Tissue factor pathway inhibitor 2; *MUC2*: mucin gene 2