Epigenetic alterations in endocrine-related cancer

Sandra Rodríguez-Rodero^{1,2}, Elías Delgado-Álvarez¹, Agustín F Fernández², Juan L Fernández-Morera¹, Edelmiro Menéndez-Torre¹ and Mario F Fraga^{2,3}

¹Endocrinology and Nutrition Service, Hospital Universitario Central de Asturias, Av. Julian Clavería s/n, 33006 Oviedo, Spain

²Cancer Epigenetics Laboratory, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Universidad de Oviedo, 33006 Oviedo, Spain

³Department of Immunology and Oncology, National Center for Biotechnology, CNB-CSIC, Cantoblanco, Madrid E-28049, Spain

Correspondence should be addressed to E Menéndez-Torre or M F Fraga
Emails
edelangot@gmail.com or mffraga@cnb.csic.es

Abstract

Aberrant epigenetics is a hallmark of cancer, and endocrine-related tumors are no exception. Recent research has been identifying an ever-growing number of epigenetic alterations in both genomic DNA methylation and histone post-translational 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, parathyroid, and breast carcinomas. 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.

Key Words

- ▶ multiple endocrine neoplasias
- ▶ neuroendocrine tumors
- ▶ oncology
- ▶ pathogenesis

Endocrine-Related Cancer
(2014) 21, R319–R330

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 focus exclusively on DNA methylation and histone modifications.

DNA methylation is the most studied epigenetic mechanism to date and occurs in ~3% of cytosines, which precede guanines as part of the so-called cytosine–guanine dinucleotides (CpGs) 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 the 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). By contrast, those CpG dinucleotides that are scattered throughout the genome are more methylated and located mainly in the bodies of genes, intergenic regions, repetitive sequences, and transposons (Martin-Subero 2011, Fedoriw et al. 2012). 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 (Fedoriw et al. 2012). It is carried out by a group of enzymes known as DNA cytosine-5-methyltransferases (DNMTs), which transfer the methyl group from S-adenosylmethionine (SAME) to the C5 position of cytosine. In mammals, five 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,' as 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 enzymes 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 histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs), 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 histone methyltransferases (HMTs), substrate specific, and employ SAME as a donor of methyl groups, while the reverse process is carried out by 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 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, ACC), islet cell tumors (gastrinoma, VIPoma, glucagonoma, and 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 (multiple endocrine neoplasia (MEN) syndromes) and those which are sporadic (thyroid, parathyroid, breast and ovarian, prostate, adrenocortical, and lung neuroendocrine tumors and pheochromocytoma, and paraganglioma).

Hereditary endocrine tumors

The MEN syndromes 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.

MEN1 syndromes (prevalence of 3/10 000) are characterized by the development of tumors of the parathyroid, 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). Germline 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, Hasani-Ranjbar et al. 2011, Delemer 2012). The protein product of *MEN1*, termed menin, is a tumor suppressor protein ubiquitously expressed in the nucleus. It has not only been reported to interact with transcription factors such as JunD, Smad3, but also been found as a component of a multiple protein complex (through interaction with the trithorax group

proteins MLL2 (KMT2B), *mixed lineage leukemia*, and MLL (KMT2A)), 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 that 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 tumors associated with MEN1 in the pancreas: seldom malignant they are derived from β -cells with an uncontrolled secretion of insulin that results in hypoglycemia (Mathur et al. 2009). The work developed by Karnik et al. (2005) demonstrated that in pancreatic endocrine cells menin interacts with *p27* (*Cdkn1b*) and *p18* (*Cdkn2c*) promoters; two cyclin-dependent kinase inhibitors that play an important role in islet growth control. Menin, through MLL2 interaction, regulates the HMT activity of this TrxG complex member and, as a transcriptional coactivator, promotes histone methylation of *p27* and *p18*. *Men1* inactivation disrupts *p27* and *p18* expression and alters islet growth control and

Table 1 Epigenetic alterations in endocrine tumors

Endocrine neoplasia	Epigenetic alteration	References
<i>Hereditary endocrine tumors</i>		
Multiple endocrine neoplasia 1 (MEN1)	Menin interaction with the trithorax proteins MLL2 and MLL, with histone H3 lysine H4 methyltransferase activity	Murai et al. (2011)
Multiple endocrine neoplasia 2 (MEN2)	Aberrant promoter methylation in <i>RASSF1A</i>	Schagdarsurengin et al. (2002)
<i>Sporadic endocrine tumors</i>		
Thyroid tumors	Aberrant promoter methylation in <i>RASSF1A</i> , <i>PTEN</i> , <i>RASSF2</i> , and <i>RASSF10</i> Aberrant promoter methylation in <i>TIMP3</i> , <i>SLC5A8</i> , and <i>DAPK</i>	Porra et al. (2005), Brzezianska & Pastuszak-Lewandoska (2011) and Brait et al. (2012)
Parathyroid tumors	Aberrant promoter methylation in <i>H1C1</i> and <i>RASSF1</i>	Juhlin et al. (2010) and Svedlund et al. (2012)
Breast cancer	Aberrant promoter methylation in <i>BRCA1</i> , <i>ERα</i> , <i>p16</i> , <i>RASSF1A</i> , and <i>SLC25A43</i>	Nass et al. (2000), Lindqvist et al. (2012), Wang et al. (2012) and Xu et al. (2012)
Ovarian cancer	Aberrant promoter methylation in <i>BRCA1</i> , <i>BRCA2</i> , <i>FHIT</i> , <i>FANCF</i> , and <i>p16</i>	Dhillon et al. (2004a,b)
Prostate cancer	Aberrant promoter methylation in <i>GSTP1</i> , <i>MGMT</i> , <i>p16</i> , <i>CDH1</i> , and <i>RASSF1A</i>	Kang et al. (2004), Verdoodt et al. (2011) and Mahapatra et al. (2012)
Pancreatic cancer	Aberrant promoter methylation in <i>p16</i> , <i>SPARC</i> , and <i>TFPI2</i> Enriched methylation in H3K9 markers of <i>MUC1</i> and <i>MUC2</i> gene promoter	Gao et al. (2010) and Park et al. (2012)
Adrenocortical cancer	Genome-wide methylation profiles	Rechache et al. (2012) and Barreau et al. (2013)

MLL2, mixed lineage leukemia; *RASSF1*, Ras-association domain family; *PTEN*, phosphatase and tensin homolog; *TIMP3*, *TIMP* metalloproteinase inhibitor; *SLC5A8*, solute carrier family; *DAPK*, death-associated protein kinase; *H1C1*, breast cancer 1; *FHIT*, fragile histidine triad protein; *FANCF*, 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); *TFPI2*, tissue factor pathway inhibitor 2; *MUC2*, mucin gene 2.

tumor suppression. Moreover, Fontanière *et al.* (2008) have demonstrated in a mice model that *Men1* disruption was not enough to trigger tumorigenesis of β -cells and that the gene expression profile of insulin-like growth factor (*Igf*) was also deregulated. They showed that *Igf2* was overexpressed in *Men1* mutant mice, as 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 54–80% of patients with MEN1 syndrome, and prolactinoma is the most common one (41–76%). Yoshino *et al.* (2007) showed promoter hypermethylation in at least one of the cell cycle regulator genes (*RB1*, *P14ARF*, *P15 (INK4b)* (*CDKN2B*), *P16 (CDKN2A)*, *P21 (CDKN1A)*, *P27*, and *P73 (TP73)*) in pituitary tumors. 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.* (2006), who found promoter hypermethylation in the *RB1*, *P14 (ARF)*, *P16*, and *P73* genes, as well as the metalloproteinase inhibitor 3 (*TIMP3*), O-6-methylguanine DNA methyltransferase (*MGMT*), *DAPK (DAPK1)*, *THBS1*, and *CASP8* genes, some of which are involved in the apoptosis processes (*DAPK* and *CASP8*), 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 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 familial medullary thyroid carcinoma (FMTC). MEN2a was 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, which are derived from the neural crest, secrete calcitonin (CTN), and glycoprotein carcinoembryonic antigen (CEA). Mutations in the *RET* proto-oncogene,

which encodes a tyrosine kinase receptor, have been described as the main cause of MEN2a, MEN2b, and FMTC.

Moreover, promoter methylation of *RASSF1A* (*RASSF1*) 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).

Sporadic endocrine diseases

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 form (50–90%), which can metastasize early in the cervical lymph nodes, and follicular thyroid carcinomas (FTCs), which spread primarily by hematogenous dissemination; the MTC, which causes early metastasis (lung and bone) originating both via the lymph and blood; and the most aggressive, anaplastic thyroid carcinomas (ATCs) with early distant metastasis. Gene promoter methylation of tumor suppressor genes such as *CDH1*, *PTEN*, *RASSF1A*, and *FGFR2* has been described as part of the pathology of the thyroid tumors (Table 1). The *PTEN* gene encodes phosphatase that blocks the signaling of the PI3K/Akt pathway, which is constitutively activated in FTCs. The *RASSF1A*, *RASSF2*, and Ras-association domain family signaling protein (*RASSF10*) genes are methylated in PTCs, FTCs, and ATCs. RAS is a component of the PI3K/Akt pathway, suggesting a relationship between PTEN and Ras proteins in the development of this pathology (Schagdarsurengin *et al.* 2009, 2010, Brzezianska & Pastuszek-Lewandoska 2011).

Other tumor suppressor genes *TIMP3*, *SLC5A8*, and *DAPK* are also aberrantly regulated by methylation in thyroid tumors (Porra *et al.* 2005, Brait *et al.* 2012). *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 PTCs. These results reveal

the importance of DNA methylation in the regulation of this gene 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 subtypes are characterized by aberrant promoter hypomethylation, which could be used in diagnosis and/or prognosis of this type of cancer (Rodríguez-Rodero et al. 2013).

Parathyroid tumors Parathyroid tumors are an abnormal growth in the parathyroid gland and usually cause hyperparathyroidism. Most of them are benign adenomas (85%) and carcinomas are very rare (0.5–1%). Overexpression of *CCND*, and deletions of the *RB1* gene or *BCRA2* genes on chromosome 13, has been identified as a possible cause of these tumors (Shattuck et al. 2003, Mallya et al. 2010).

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 downmodulation 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 thyroid tumors, promoter hypermethylation in *RASSF1* 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).

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 has also been suggested as a prognosis factor as 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 cancers.

Many breast cancers are sensitive to the hormone estrogen, which can contribute to tumor development. Those cancers with estrogen receptors (ERs) on the surface of the cancer cells are termed 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 ERs, *ERa* and *ERb*, which are encoded by the genes *ESR1* and *ESR2*, respectively, and function as transcription factors to regulate gene expression. *ERa* is expressed in the great majority of breast tumors (75%) (*ERa*-positive) and frequently associated with a better prognosis and responsiveness to hormone treatment, while *ERa*-negative breast tumors are associated with a poorer prognosis and a greater malignancy (Xie et al. 2012). One mechanism involved in suppressing *ERa* expression in ER-negative tumors is aberrant methylation of CpG islands at the *ERa* promoter, which is present more frequently in metastatic tumors (associated with adverse clinical outcomes) 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 metastasis tumor antigen 1 (*MTA1*) gene (observed when breast cancer-invasive cell lines were 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 is an

enrichment of H3K9me3 that acts as a repressive marker of transcription. Upon 17 β -estradiol activation, JMJD2B expression is induced, and it is recruited to ER α target sites and demethylates H3K9me3. Moreover, this interaction with the receptor also allows the recruitment of histone-modifying enzymes, such as 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 solute carrier family 25A member 43 (*SLC25A43*) 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 the diagnosis of 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. (2012) have demonstrated higher methylation in *BCAN*, *HOXD1*, *KCTD8*, *KLF11*, *NXPH1*, *POU4F1*, *SIM1*, and *TCF7L1* genes in low-grade breast tumors. These results were confirmed by the findings of van Hoesel et al. (2013), which demonstrated an increase in promoter methylation in methylated-IN-tumor 17 (*MINT17*), *MINT31*, *RAR β 2* (*RARB*), and *RASSF1A* genes, associated with a poor prognosis of the disease.

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. They produce androgens (male hormones) less frequently. Dhillon et al. found promoter methylation in a group of genes: *FHIT* (28%), a tumor suppressor gene also hypermethylated in other tumor subtypes; *FANCF* (24%), which interacts with *BRCA1* and *BRCA2* pathways; *CCND2* (12%), involved in the regulation of transition from G1-phase to S-phase 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 the regulation of the cell cycle during proliferation and differentiation; and *RUNX3* (56%), which belongs to the *RUNT* domain genes and also plays a tumor suppressor role (Dhillon et al. 2004a; Table 1).

Dhillon et al. (2004b) also showed promoter hypermethylation in *P16*, *BRCA1*, *RASSF1A*, *ER α* (*ESR1*), *TMS1* (*PYCARD*), *TIMP3*, *TWIST* (*TWIST1*), *GSTP1*, androgen receptor (*AR*), and human *MLH1* genes in ovarian cancer. *TIMP3*, 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 (Schagdarsurengin et al. 2009, 2010, Buckingham et al. 2010, Brzezianska & Pastuszak-Lewandoska 2011). *P16* silencing, by this epigenetic mechanism, seems to be an early event in the development and progression of ovarian cancers (Dhillon et al. 2004b).

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 and excess weight), hormonal factors (androgens play an important role in the initiation and promotion of prostate cancer), and genetic factors such as the *BRCA2* gene or *ELAC2* (individuals with affected relatives, respectively, have a 3.1 and 4.3 times higher risk when compared with healthy controls) (Wallner et al. 2011). The majority of prostate tumors are adenocarcinomas, while <1% are neuroendocrine tumors (Cohen 2005). Valentini et al. (2007) demonstrated that when human prostate androgen-dependent cancer cell line LNCaP was treated with valproic acid, an inhibitor of HDACs, this component induced neuroendocrine-like differentiation in this cell line as well as downregulation of AR protein and reduction in prostate-specific antigen. Moreover, prostate cancer cells commonly exhibit promoter hypermethylation, which causes gene repression in the acquisition and maintenance of the neoplastic phenotype. *GSTP1* is the most frequently methylated gene in prostate cancer. Glutathione S-transferases (GSTs) comprise a family of enzymes involved in the detoxification of xenobiotics and oxygen radicals. Absent or diminished expression of the *GSTP1* gene in prostate cancer has been reported and is commonly due to CpG island *GSTP1* promoter region methylation in the majority of prostate tumors (>90%), a phenomenon that is rarely detected in normal prostate or benign prostatic hyperplasia tissues. This gene has also been described as hypermethylated in breast carcinoma, an early event, which suggests its utility as a biomarker (Mahapatra et al. 2012, Saxena et al. 2012, Fukushige &

Horii 2013, Song *et al.* 2013). The DNA alkyl-repair gene *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 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 ERs 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, such as the tumor suppressor gene *P16*, a cyclin-dependent kinase inhibitor (Verdoodt *et al.* 2011), or *RASSF1A* (found in 49–99% of tumors), mentioned in earlier sections, which are also associated with other tumor types and deregulation of which 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 & Banerjee 2012). Another hypermethylated tumor suppressor gene in prostate cancer is adenomatous polyposis coli (*APC*), 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*, *CD44*, and *TIMP3*) 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 (*TIMP3*), 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 & van Helden 1987) and could contribute to tumoral transformation through multiple oncogene activation (i.e., *MYC* and *HRAS*) and by chromosome instability.

Among the post-translational 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 cancer patients (Wissmann *et al.* 2007, Wang *et al.* 2009).

Enhancer of zeste homolog 2 (*EZH2*) has been found overexpressed in prostate cancer, with higher expression in those that are metastatic. This protein is a member of the polycomb repressive complex 2 (*PRC2*), which causes trimethylation of histone H3 on Lys27 (*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 such as *ADRB2*, *CDH1*, *PSP94* (*MSMB*), and *DAB2IP* (Ren *et al.* 2012). Similarly, Babbio *et al.* (2012) 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. 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.

Pancreatic cancer Pancreatic neoplasms arise from the endocrine and exocrine portions of the organ, where the most frequent forms 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 tumor (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.* (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).

Adrenocortical carcinomas Adrenocortical cancer is rare (0.5–2 cases per million) though very aggressive. The majority of tumors are functional (60% of patients) (Lafemina & Brennan 2012). Non-functional tumors (40%) present as incidentalomas, frequently affect older people, and have poorer prognosis; they do not secrete any hormones or present specific symptoms (Lehmann & Wrzesinski 2012).

The first genome-wide DNA methylation profiling in adrenocortical tumors (ACTs) 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 (Rodríguez-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 downregulated genes in ACCs were also identified (Rechache et al. 2012). Similarly, Fonseca et al. (2012) conducted genome-wide methylation studies, finding 212 CpG islands in promoter regions that were significantly hypermethylated and that might contribute to pathology development. Barreau et al. (2013) focused their analysis on promoter CpG islands of exclusively tumoral samples (84 adrenocortical adenomas and 51 ACCs) and found that ACCs were more hypermethylated than adenomas, in accordance with previous results. They also described that ACCs could be subdivided into samples which are slightly more methylated (non-CIMP) and those that are highly methylated (CIMP). Finally, Suh et al. (2010) have demonstrated that treatment of ACTs with decitabine (5-aza-2'-deoxycytidine) reverses DNA promoter methylation of those genes with an antitumoral function (Table 1).

Pheochromocytomas and paraganglioma Pheochromocytoma usually arises from the adrenal medulla. It is a catecholamine (epinephrine and norepinephrine) producing tumor arising from chromaffin cells derived from the sympathetic nervous system. Pheochromocytomas can also develop extra-adrenally (from chromaffin cells), when they are called paragangliomas. Both tumor types mostly occur sporadically, although the hereditary

syndromes, MEN2a and 2b, von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1), and paraganglioma syndromes (PGLs) 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* predispose to tumor development in these disorders. Recently, Sandgren et al. (2010) have found an increase in *EZH2* expression in tumor samples when compared with normal adrenal medulla. The upregulation 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 cancer 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 (Krajnovic et al. 2013, Pierini et al. 2013). However, this hypermethylation was not observed in *MEN2/RET*-associated paragangliomas (Kiss et al. 2008, 2013, Muscarella et al. 2008).

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. (2012) found a group of 73 genes to be aberrantly methylated in more than 77% of primary SCLC tumors. 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), have 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 have revealed the presence of certain genes with a tumor suppressor role where the promoter is aberrantly methylated, which may contribute to 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 for the possibility of interventions to reverse such changes in order to treat the disease or arrest its development.

Although there are many types of endocrine tumors and, for some, only a small number of candidate genes have been analyzed, we have described some common epigenetic alterations to both hereditary and sporadic endocrine tumors in this review (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 that 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 prejudicing the impartiality of the review reported.

Funding

This work was financially supported by the Fundación Asturcor (to S Rodríguez-Rodero), the Spanish National Research Council (CSIC; 2008201172 to M F Fraga), Fundación Ramón Areces (to M F Fraga), the Fondo de Investigaciones Sanitarias FIS/FEDER (PI11/01728 to

A F Fernández, PI11/02795 to E D Delgado-Álvarez, and PI12/01080 to M F Fraga), the PN de I+D+I 2008-20011 and the ISCIII-Subdirección General de Evaluación y Fomento de la Investigación (CP11/00131 to A F Fernández), Fundación Mutua Madrileña (to E Menéndez-Torre), and Sociedad española de diabetes (to J L Fernández-Morera). The IUOPA was supported by the Obra Social Cajastur, Spain.

Acknowledgements

The authors thank Ronnie Lendrum for editorial assistance.

References

- Abou-Zeid AA, Azzam AZ & Kamel NA 2011 Methylation status of the gene promoter of cyclin-dependent kinase inhibitor 2A (CDKN2A) in ovarian cancer. *Scandinavian Journal of Clinical and Laboratory Investigation* **71** 542–547. (doi:10.3109/00365513.2011.590224)
- Amin KS & Banerjee PP 2012 The cellular functions of RASSF1A and its inactivation in prostate cancer. *Journal of Carcinogenesis* **11** 3. (doi:10.4103/1477-3163.93000)
- Anania MC, Sensi M, Radaelli E, Miranda C, Vizioli MG, Pagliardini S, Favini E, Cleris L, Supino R, Formelli F *et al.* 2011 TIMP3 regulates migration, invasion and *in vivo* tumorigenicity of thyroid tumor cells. *Oncogene* **30** 3011–3023. (doi:10.1038/ncr.2011.18)
- Antequera F 2003 Structure, function and evolution of CpG island promoters. *Cellular and Molecular Life Sciences* **60** 1647–1658. (doi:10.1007/s00018-003-3088-6)
- Babbio F, Pistore C, Curti L, Castiglioni I, Kunderfranco P, Brino L, Oudet P, Seiler R, Thalman GN, Roggero E *et al.* 2012 The SRA protein UHRF1 promotes epigenetic crosstalks and is involved in prostate cancer progression. *Oncogene* **31** 4878–4887. (doi:10.1038/ncr.2011.641)
- Bammidi LS, Neerukonda GN, Murthy S & Kanapuram RD 2012 p16 gene alterations in human ovarian cancers: comparison between tissue and blood samples. *International Journal of Gynecological Cancer* **22** 553–560. (doi:10.1097/IGC.0b013e31823fa90c)
- Barreau O, Assie G, Wilmot-Roussel H, Ragazzon B, Baudry C, Perlemoine K, Rene-Corail F, Bertagna X, Dousset B, Hamzaoui N *et al.* 2013 Identification of a CpG island methylator phenotype in adrenocortical carcinomas. *Journal of Clinical Endocrinology and Metabolism* **98** E174–E184. (doi:10.1210/jc.2012-2993)
- Bedford MT & van Helden PD 1987 Hypomethylation of DNA in pathological conditions of the human prostate. *Cancer Research* **47** 5274–5276.
- Bediaga NG, Acha-Sagredo A, Guerra I, Viguri A, Albaina C, Ruiz Daiz I, Rezola R, Alberdi MJ, Dopazo J, Montaner D *et al.* 2010 DNA methylation epigenotypes in breast cancer molecular subtypes. *Breast Cancer Research* **12** R77. (doi:10.1186/bcr2721)
- Bello MJ, De Campos JM, Isla A, Casartelli C & Rey JA 2006 Promoter CpG methylation of multiple genes in pituitary adenomas: frequent involvement of caspase-8. *Oncology Reports* **15** 443–448.
- Berstein LM, Poroshina TE, Vasilyev DA & Boyarkina MP 2010 Evaluation of the proportion of hormonal and progenotoxic effects of estrogens and glucose in cancer patients. *Bulletin of Experimental Biology and Medicine* **150** 243–246. (doi:10.1007/s10517-010-1115-9)
- Bird A 2002 DNA methylation patterns and epigenetic memory. *Genes and Development* **16** 6–21. (doi:10.1101/gad.947102)
- Bosviel R, Garcia S, Lavediaux G, Michard E, Dravers M, Kwiatkowski F, Bignon YJ & Bernard-Gallon DJ 2012 BRCA1 promoter methylation in peripheral blood DNA was identified in sporadic breast cancer and controls. *Cancer Epidemiology* **36** e177–e182. (doi:10.1016/j.canep.2012.02.001)
- Brait M, Loyo M, Rosenbaum E, Ostrow KL, Markova A, Papagerakis S, Zahurak M, Goodman SM, Zeiger M, Sidransky D *et al.* 2012 Correlation

- between BRAF mutation and promoter methylation of TIMP3, RAR β 2 and RASSF1A in thyroid cancer. *Epigenetics* **7** 710–719. (doi:10.4161/epi.20524)
- Brzezianska E & Pastuszak-Lewandoska D 2011 A minireview: the role of MAPK/ERK and PI3K/Akt pathways in thyroid follicular cell-derived neoplasm. *Frontiers in Bioscience* **16** 422–439. (doi:10.2741/3696)
- Buckingham L, Penfield Faber L, Kim A, Liptay M, Barger C, Basu S, Fidler M, Walters K, Bonomi P & Coon J 2010 PTEN, RASSF1 and DAPK site-specific hypermethylation and outcome in surgically treated stage I and II nonsmall cell lung cancer patients. *International Journal of Cancer* **126** 1630–1639. (doi:10.1002/ijc.24896)
- Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA et al. 1997 Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* **276** 404–407. (doi:10.1126/science.276.5311.404)
- Chang PY, Hom RA, Musselman CA, Zhu L, Kuo A, Gozani O, Kutateladze TG & Cleary ML 2011 Binding of the MLL PHD3 finger to histone H3K4me3 is required for MLL-dependent gene transcription. *Journal of Molecular Biology* **400** 137–144. (doi:10.1016/j.jmb.2010.05.005)
- Chedin F 2011 The DNMT3 family of mammalian *de novo* DNA methyltransferases. *Progress in Molecular Biology and Translational Science* **101** 255–285. (doi:10.1016/B978-0-12-387685-0.00007-X)
- Cohen RJ 2005 Neuroendocrine carcinoma of the prostate gland. *Pathology* **37** 326–327. (doi:10.1080/00313020500169420)
- Deb G, Thakur VS & Gupta S 2013 Multifaceted role of EZH2 in breast and prostate tumorigenesis: epigenetics and beyond. *Epigenetics* **8** 464–476. (doi:10.4161/epi.24532)
- Debelenko LV, Swallowell JI, Kelley MJ, Brambilla E, Manickam P, Baibakov G, Agarwal SK, Spiegel AM, Marx SJ, Chandrasekharappa SC et al. 2000 MEN1 gene mutation analysis of high-grade neuroendocrine lung carcinoma. *Genes, Chromosomes & Cancer* **28** 58–65. (doi:10.1002/(SICI)1098-2264(200005)28:1<58::AID-GCC7>3.0.CO;2-2)
- Delemer B 2012 MEN1 and pituitary adenomas. *Annales d'Endocrinologie* **73** 59–61. (doi:10.1016/j.ando.2012.03.038)
- Delgado-Cruzata L, Hruby GW, Gonzalez K, McKiernan J, Benson MC, Santella RM & Shen J 2012 DNA methylation changes correlate with Gleason score and tumor stage in prostate cancer. *DNA and Cell Biology* **31** 187–192. (doi:10.1089/dna.2011.1311)
- Dhillon VS, Shahid M & Husain SA 2004a CpG methylation of the FHIT, FANCF, cyclin-D2, BRCA2 and RUNX3 genes in granulosa cell tumors (GCTs) of ovarian origin. *Molecular Cancer* **3** 33. (doi:10.1186/1476-4598-3-33)
- Dhillon VS, Aslam M & Husain SA 2004b The contribution of genetic and epigenetic changes in granulosa cell tumors of ovarian origin. *Clinical Cancer Research* **10** 5537–5545. (doi:10.1158/1078-0432.CCR-04-0228)
- Faryna M, Konermann C, Aulmann S, Bermejo JL, Brugger M, Diederichs S, Rom J, Weichenhan D, Claus R, Rehli M et al. 2012 Genome-wide methylation screen in low-grade breast cancer identifies novel epigenetically altered genes as potential biomarkers for tumor diagnosis. *FASEB Journal* **26** 4937–4950. (doi:10.1096/fj.12-209502)
- Fatemi M, Hermann A, Gowher H & Jeltsch A 2002 Dnmt3a and Dnmt1 functionally cooperate during *de novo* methylation of DNA. *European Journal of Biochemistry* **269** 4981–4984. (doi:10.1046/j.1432-1033.2002.03198.x)
- Fedorow A, Mugford J & Magnuson T 2012 Genomic imprinting and epigenetic control of development. *Cold Spring Harbor Perspectives in Biology* **4** a008136. (doi:10.1101/cshperspect.a008136)
- Fernandez AF, Huidobro C & Fraga MF 2012 *De novo* DNA methyltransferases: oncogenes, tumor suppressors, or both? *Trends in Genetics* **28** 474–479. (doi:10.1016/j.tig.2012.05.006)
- Fontanière S, Duvillie B, Scharfmann R, Carreira C, Wang ZQ & Zhang CX 2008 Tumour suppressor menin is essential for development of the pancreatic endocrine cells. *Journal of Endocrinology* **199** 287–298. (doi:10.1677/JOE-08-0289)
- Fonseca AL, Kugelberg J, Starker LF, Scholl U, Choi M, Hellman P, Akerstrom G, Westin G, Lifton RP, Bjorklund P et al. 2012 Comprehensive DNA methylation analysis of benign and malignant adrenocortical tumors. *Genes, Chromosomes & Cancer* **51** 949–960. (doi:10.1002/gcc.21978)
- Fukushige S & Horii A 2013 DNA methylation in cancer: a gene silencing mechanism and the clinical potential of its biomarkers. *Tohoku Journal of Experimental Medicine* **229** 173–185. (doi:10.1620/tjem.229.173)
- Gao J, Song J, Huang H, Li Z, Du Y, Cao J, Li M, Lv S, Lin H & Gong Y 2010 Methylation of the SPARC gene promoter and its clinical implication in pancreatic cancer. *Journal of Experimental & Clinical Cancer Research* **29** 28. (doi:10.1186/1756-9966-29-28)
- Hasani-Ranjbar S, Amoli MM, Ebrahim-Habibi A, Gozashti MH, Khalili N, Sayyahpour FA, Hafeziyeh J, Soltani A & Larijani B 2011 A new frameshift MEN1 gene mutation associated with familial malignant insulinomas. *Familial Cancer* **10** 343–348. (doi:10.1007/s10689-010-9412-z)
- Hermann A, Gowher H & Jeltsch A 2004 Biochemistry and biology of mammalian DNA methyltransferases. *Cellular and Molecular Life Sciences* **61** 2571–2587. (doi:10.1007/s00018-004-4201-1)
- van Hoesel AQ, Sato Y, Elashoff DA, Turner RR, Giuliano AE, Shamonki JM, Kuppen PJ, van de Velde CJ & Hoon DS 2013 Assessment of DNA methylation status in early stages of breast cancer development. *British Journal of Cancer* **108** 2033–2038. (doi:10.1038/bjc.2013.136)
- Hoog A, Kjellman M, Nordqvist AC, Hoog CM, Juhlin C, Falkmer S, Schalling M & Grimelius L 2001 Insulin-like growth factor-II in endocrine pancreatic tumours. Immunohistochemical, biochemical and *in situ* hybridization findings. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica* **109** 127–140. (doi:10.1034/j.1600-0463.2001.d01-114.x)
- Huang Y, Nayak S, Jankowitz R, Davidson NE & Oesterreich S 2011 Epigenetics in breast cancer: what's new? *Breast Cancer Research* **13** 225. (doi:10.1186/bcr2925)
- Jeronimo C, Henrique R, Hoque MO, Mambo E, Ribeiro FR, Varzim G, Oliveira J, Teixeira MR, Lopes C & Sidransky D 2004 A quantitative promoter methylation profile of prostate cancer. *Clinical Cancer Research* **10** 8472–8478. (doi:10.1158/1078-0432.CCR-04-0894)
- Juhlin CC, Kiss NB, Villablanca A, Haglund F, Nordenstrom J, Hoog A & Larsson C 2010 Frequent promoter hypermethylation of the APC and RASSF1A tumour suppressors in parathyroid tumours. *PLoS ONE* **5** e9472. (doi:10.1371/journal.pone.0009472)
- Kalari S, Jung M, Kernstine KH, Takahashi T & Pfeifer GP 2012 The DNA methylation landscape of small cell lung cancer suggests a differentiation defect of neuroendocrine cells. *Oncogene* **32** 3559–3568. (doi:10.1038/onc.2012.362)
- Kang GH, Lee S, Lee HJ & Hwang KS 2004 Aberrant CpG island hypermethylation of multiple genes in prostate cancer and prostatic intraepithelial neoplasia. *Journal of Pathology* **202** 233–240. (doi:10.1002/path.1503)
- Karnik SK, Hughes CM, Gu X, Rozenblatt-Rosen O, McLean GW, Xiong Y, Meyerson M & Kim SK 2005 Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27Kip1 and p18INK4c. *PNAS* **102** 14659–14664. (doi:10.1073/pnas.0503484102)
- Kiss NB, Geli J, Lundberg F, Avci C, Velazquez-Fernandez D, Hashemi J, Weber G, Hoog A, Ekstrom TJ, Backdahl M et al. 2008 Methylation of the p16INK4A promoter is associated with malignant behavior in abdominal extra-adrenal paragangliomas but not pheochromocytomas. *Endocrine-Related Cancer* **15** 609–621. (doi:10.1677/ERC-07-0285)
- Kiss NB, Muth A, Andreasson A, Juhlin CC, Geli J, Backdahl M, Hoog A, Wangberg B, Nilsson O, Ahlman H et al. 2013 Acquired hypermethylation of the P16INK4A promoter in abdominal paraganglioma: relation to adverse tumor phenotype and predisposing mutation. *Endocrine-Related Cancer* **20** 65–78. (doi:10.1530/ERC-12-0267)

- Krajnovic M, Radojkovic M, Davidovic R, Dimitrijevic B & Krtolica K 2013 Prognostic significance of epigenetic inactivation of p16, p15, MGMT and DAPK genes in follicular lymphoma. *Medical Oncology* **30** 441. (doi:10.1007/s12032-012-0441-3)
- Lafemina J & Brennan MF 2012 Adrenocortical carcinoma: past, present, and future. *Journal of Surgical Oncology* **106** 586–594. (doi:10.1002/jso.23112)
- Lehmann T & Wrzesinski T 2012 The molecular basis of adrenocortical cancer. *Cancer Genetics* **205** 131–137. (doi:10.1016/j.cancergen.2012.02.009)
- Li LC, Chui R, Nakajima K, Oh BR, Au HC & Dahiya R 2000 Frequent methylation of estrogen receptor in prostate cancer: correlation with tumor progression. *Cancer Research* **60** 702–706.
- Li LC, Zhao H, Nakajima K, Oh BR, Ribeiro Filho LA, Carroll P & Dahiya R 2001 Methylation of the E-cadherin gene promoter correlates with progression of prostate cancer. *Journal of Urology* **166** 705–709. (doi:10.1016/S0022-5347(05)66047-8)
- Li F, Ye B, Hong L, Xu H & Fishbein MC 2011 Epigenetic modifications of histone h4 in lung neuroendocrine tumors. *Applied Immunohistochemistry & Molecular Morphology* **19** 389–394. (doi:10.1097/PAI.0b013e3182108e2e)
- Li KK, Luo C, Wang D, Jiang H & Zheng YG 2012 Chemical and biochemical approaches in the study of histone methylation and demethylation. *Medicinal Research Reviews* **32** 815–867. (doi:10.1002/mrr.20228)
- Lindqvist BM, Farkas SA, Wingren S & Nilsson TK 2012 DNA methylation pattern of the SLC25A43 gene in breast cancer. *Epigenetics* **7** 300–306. (doi:10.4161/epi.7.3.19064)
- Lister R, Pelizzola M, Downen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM et al. 2009 Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462** 315–322. (doi:10.1038/nature08514)
- Lou W, Krill D, Dhir R, Becich MJ, Dong JT, Frierson HF Jr, Isaacs WB, Isaacs JT & Gao AC 1999 Methylation of the CD44 metastasis suppressor gene in human prostate cancer. *Cancer Research* **59** 2329–2331.
- Mahapatra S, Klee EW, Young CY, Sun Z, Jimenez RE, Klee GG, Tindall DJ & Donkena KV 2012 Global methylation profiling for risk prediction of prostate cancer. *Clinical Cancer Research* **18** 2882–2895. (doi:10.1158/1078-0432.CCR-11-2090)
- Mallya SM, Wu HI, Saria EA, Corrado KR & Arnold A 2010 Tissue-specific regulatory regions of the PTH gene localized by novel chromosome 11 rearrangement breakpoints in a parathyroid adenoma. *Journal of Bone and Mineral Research* **25** 2606–2612. (doi:10.1002/jbmr.187)
- Mao XY, Chen H, Wang H, Wei J, Liu C, Zheng HC, Yao F & Jin F 2012 MTA1 expression correlates significantly with ER- α methylation in breast cancer. *Tumor Biology* **33** 1565–1572. (doi:10.1007/s13277-012-0410-7)
- Martin-Subero JI 2011 How epigenomics brings phenotype into being. *Pediatric Endocrinology Reviews* **9** (Suppl 1) 506–510.
- Mathur A, Gorden P & Libutti SK 2009 Insulinoma. *Surgical Clinics of North America* **89** 1105–1121. (doi:10.1016/j.suc.2009.06.009)
- Murai MJ, Chruszcz M, Reddy G, Grembecka J & Cierpicki T 2011 Crystal structure of menin reveals binding site for mixed lineage leukemia (MLL) protein. *Journal of Biological Chemistry* **286** 31742–31748. (doi:10.1074/jbc.M111.258186)
- Muscarella P, Bloomston M, Brewer AR, Mahajan A, Frankel WL, Ellison EC, Farrar WB, Weghorst CM & Li J 2008 Expression of the p16INK4A/Cdkn2a gene is prevalently downregulated in human pheochromocytoma tumor specimens. *Gene Expression* **14** 207–216. (doi:10.3727/105221608786883825)
- Nakayama T, Watanabe M, Suzuki H, Toyota M, Sekita N, Hirokawa Y, Mizokami A, Ito H, Yatani R & Shiraishi T 2000 Epigenetic regulation of androgen receptor gene expression in human prostate cancers. *Laboratory Investigation* **80** 1789–1796. (doi:10.1038/labinvest.3780190)
- Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, Davidson NE & Graff JR 2000 Aberrant methylation of the estrogen receptor and E-cadherin 5' CpG islands increases with malignant progression in human breast cancer. *Cancer Research* **60** 4346–4348.
- Pakala SB, Rayala SK, Wang RA, Ohshiro K, Mudvari P, Reddy SD, Zheng Y, Pires R, Casimiro S, Pillai MR et al. 2013 MTA1 promotes STAT3 transcription and pulmonary metastasis in breast cancer. *Cancer Research* **73** 3761–3770. (doi:10.1158/0008-5472.CAN-12-3998)
- Park JW, Baek IH & Kim YT 2010 Preliminary study analyzing the methylated genes in the plasma of patients with pancreatic cancer. *Scandinavian Journal of Surgery* **101** 38–44.
- Pierini S, Jordanov SH, Mitkova AV, Chalakov IJ, Melnicharov MB, Kunev KV, Mitev VI, Kaneva RP & Goranova TE 2013 Promoter hypermethylation of CDKN2A, MGMT, MLH1 and DAPK genes in laryngeal squamous cell carcinoma and their associations with clinical profiles of the patients. *Head & Neck* [in press]. (doi:10.1002/hed.23413)
- Porra V, Ferraro-Peyret C, Durand C, Selmi-Ruby S, Giroud H, Berger-Dutrieux N, Decaussin M, Peix JL, Bournaud C, Orgiazzi J et al. 2005 Silencing of the tumor suppressor gene SLC5A8 is associated with BRAF mutations in classical papillary thyroid carcinomas. *Journal of Clinical Endocrinology and Metabolism* **90** 3028–3035. (doi:10.1210/jc.2004-1394)
- Qin W, Leonhardt H & Pichler G 2011 Regulation of DNA methyltransferase 1 by interactions and modifications. *Nucleus* **2** 392–402. (doi:10.4161/nucl.2.5.17928)
- Rechache NS, Wang Y, Stevenson HS, Killian JK, Edelman DC, Merino M, Zhang L, Nilubol N, Stratakis CA, Meltzer PS et al. 2012 DNA methylation profiling identifies global methylation differences and markers of adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* **97** E1004–E1013. (doi:10.1210/jc.2011-3298)
- Ren G, Baritaki S, Marathe H, Feng J, Park S, Beach S, Bazeley PS, Beshir AB, Fenteany G, Mehra R et al. 2012 Polycomb protein EZH2 regulates tumor invasion via the transcriptional repression of the metastasis suppressor RKIP in breast and prostate cancer. *Cancer Research* **72** 3091–3104. (doi:10.1158/0008-5472.CAN-11-3546)
- Rodríguez-Rodero S, Fernandez AF, Fernandez-Morera JL, Castro-Santos P, Bayon GF, Ferrero C, Urduño RG, Gonzalez-Marquez R, Suarez C, Fernandez-Vega I et al. 2013 DNA methylation signatures identify biologically distinct thyroid cancer subtypes. *Journal of Clinical Endocrinology and Metabolism* **98** 2811–2821. (doi:10.1210/jc.2012-3566)
- Sandgren J, Andersson R, Rada-Iglesias A, Enroth S, Akerstrom G, Dumanski JP, Komorowski J, Westin G & Wadelius C 2010 Integrative epigenomic and genomic analysis of malignant pheochromocytoma. *Experimental and Molecular Medicine* **42** 484–502. (doi:10.3858/emmm.2010.42.7.050)
- Saxena A, Dhillon VS, Shahid HS, Rani M, Prasad DT, Hedau S, Hussain A, Naqvi RA, Deo SV et al. 2012 GSTP1 methylation and polymorphism increase the risk of breast cancer and the effects of diet and lifestyle in breast cancer patients. *Experimental and Therapeutic Medicine* **4** 1097–1103.
- Schagdarsurengin U, Gimm O, Hoang-Vu C, Dralle H, Pfeifer GP & Dammann R 2002 Frequent epigenetic silencing of the CpG island promoter of RASSF1A in thyroid carcinoma. *Cancer Research* **62** 3698–3701.
- Schagdarsurengin U, Richter AM, Wohler C & Dammann RH 2009 Frequent epigenetic inactivation of RASSF10 in thyroid cancer. *Epigenetics* **4** 571–576. (doi:10.4161/epi.4.8.10056)
- Schagdarsurengin U, Richter AM, Hornung J, Lange C, Steinmann K & Dammann RH 2010 Frequent epigenetic inactivation of RASSF2 in thyroid cancer and functional consequences. *Molecular Cancer* **9** 264. (doi:10.1186/1476-4598-9-264)
- Serrano J, Goebel SU, Peghini PL, Lubensky IA, Gibril F & Jensen RT 2000 Alterations in the p16INK4a/CDKN2A tumor suppressor gene in

- gastrinomas. *Journal of Clinical Endocrinology and Metabolism* **85** 4146–4156. (doi:10.1210/jcem.85.11.6970)
- Shattuck TM, Kim TS, Costa J, Yandell DW, Imanishi Y, Palanisamy N, Gaz RD, Shoback D, Clark OH, Monchik JM *et al.* 2003 Mutational analyses of RB and BRCA2 as candidate tumour suppressor genes in parathyroid carcinoma. *Clinical Endocrinology* **59** 180–189. (doi:10.1046/j.1365-2265.2003.01814.x)
- Shi L, Sun L, Li Q, Liang J, Yu W, Yi X, Yang X, Li Y, Han X, Zhang Y *et al.* 2011 Histone demethylase JMJD2B coordinates H3K4/H3K9 methylation and promotes hormonally responsive breast carcinogenesis. *PNAS* **108** 7541–7546. (doi:10.1073/pnas.1017374108)
- Shi J, Zhang G, Yao D, Liu W, Wang N, Ji M, He N, Shi B & Hou P 2012 Prognostic significance of aberrant gene methylation in gastric cancer. *American Journal of Cancer Research* **2** 116–129.
- Shinojima T, Yu Q, Huang SK, Li M, Mizuno R, Liu ET, Hoon DS & Lessard L 2012 Heterogeneous epigenetic regulation of TIMP3 in prostate cancer. *Epigenetics* **7** 1279–1289. (doi:10.4161/epi.22333)
- Song MA, Tiirikainen M, Kwee S, Okimoto G, Yu H & Wong LL 2013 Elucidating the landscape of aberrant DNA methylation in hepatocellular carcinoma. *PLoS ONE* **8** e55761. (doi:10.1371/journal.pone.0055761)
- Starker LF, Svedlund J, Udelsman R, Dralle H, Akerstrom G, Westin G, Lifton RP, Bjorklund P & Carling T 2011 The DNA methylome of benign and malignant parathyroid tumors. *Genes, Chromosomes & Cancer* **50** 735–745. (doi:10.1002/gcc.20895)
- Suh I, Weng J, Fernandez-Ranvier G, Shen WT, Duh QY, Clark OH & Kebebew E 2010 Antineoplastic effects of decitabine, an inhibitor of DNA promoter methylation, in adrenocortical carcinoma cells. *Archives of Surgery* **145** 226–232. (doi:10.1001/archsurg.2009.292)
- Sulaiman L, Juhlin CC, Nilsson IL, Fotouhi O, Larsson C & Hashemi J 2013 Global and gene-specific promoter methylation analysis in primary hyperparathyroidism. *Epigenetics* **8** 646–655. (doi:10.4161/epi.24823)
- Svedlund J, Koskinen Edblom S, Marquez VE, Akerstrom G, Bjorklund P & Westin G 2012 Hypermethylated in cancer 1 (HIC1), a tumor suppressor gene epigenetically deregulated in hyperparathyroid tumors by histone H3 lysine modification. *Journal of Clinical Endocrinology and Metabolism* **97** E1307–E1315. (doi:10.1210/jc.2011-3136)
- Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, Melmed S, Sakurai A, Tonelli F & Brandi ML 2012 Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *Journal of Clinical Endocrinology and Metabolism* **97** 2990–3011. (doi:10.1210/jc.2012-1230)
- Thiel AT, Huang J, Lei M & Hua X 2012 Menin as a hub controlling mixed lineage leukemia. *Bioessays* **34** 771–780.
- Valentini A, Biancolella M, Amati F, Gravina P, Miano R, Chillemi G, Farcomeni A, Bueno S, Vespasiani G, Desideri A *et al.* 2007 Valproic acid induces neuroendocrine differentiation and UGT2B7 up-regulation in human prostate carcinoma cell line. *Drug Metabolism and Disposition* **35** 968–972. (doi:10.1124/dmd.107.014662)
- Vasavada RC, Gonzalez-Pertusa JA, Fujinaka Y, Fiaschi-Taesch N, Cozar-Castellano I & Garcia-Ocana A 2006 Growth factors and β cell replication. *International Journal of Biochemistry & Cell Biology* **38** 931–950.
- Verdoordt B, Sommerer F, Palisaar RJ, Noldus J, Vogt M, Nambiar S, Tannapfel A, Mirmohammadsadegh A & Neid M 2011 Inverse association of p16 INK4a and p14 ARF methylation of the CDKN2a locus in different Gleason scores of prostate cancer. *Prostate Cancer and Prostatic Diseases* **14** 295–301. (doi:10.1038/pcan.2011.45)
- Waddington C 1942 The epigenotype. *Endeavour* **1** 18–20.
- Wallner LP, Morgenstern H, McGree ME, Jacobson DJ, St Sauver JL, Jacobsen SJ & Sarma AV 2011 The effects of metabolic conditions on prostate cancer incidence over 15 years of follow-up: results from the Olmsted County Study. *BJU International* **107** 929–935. (doi:10.1111/j.1464-410X.2010.09703.x)
- Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, Chen Z, Beroukhim R, Wang H, Lupien M *et al.* 2009 Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. *Cell* **138** 245–256. (doi:10.1016/j.cell.2009.04.056)
- Wang L, Tang L, Xie R, Nie W, Chen L & Guan X 2012 p16 promoter hypermethylation is associated with increased breast cancer risk. *Molecular Medicine Reports* **6** 904–908. (doi:10.3892/mmr.2012.1001)
- Wang YQ, Yan Q, Zhang JR, Li SD, Yang YX & Wan XP 2013 Epigenetic inactivation of BRCA1 through promoter hypermethylation in ovarian cancer progression. *Journal of Obstetrics and Gynaecology Research* **39** 549–554. (doi:10.1111/j.1447-0756.2012.01979REF98=10.1186/1471-2407-12-243)
- Wissmann M, Yin N, Muller JM, Greschik H, Fodor BD, Jenuwein T, Vogler C, Schneider R, Gunther T, Buettner R *et al.* 2007 Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nature Cell Biology* **9** 347–353.
- Xie M, Zhu X, Liu Z, Shrubsole M, Varma V, Mayer IA, Dai Q, Chen Q & You S 2012 Membrane progesterone receptor α as a potential prognostic biomarker for breast cancer survival: a retrospective study. *PLoS ONE* **7** e35198. (doi:10.1371/journal.pone.0035198)
- Xu J, Shetty PB, Feng W, Chenault C, Bast RC Jr, Issa JP, Hilsenbeck SG & Yu Y 2012 Methylation of HIN-1, RASSF1A, RIL and CDH13 in breast cancer is associated with clinical characteristics, but only RASSF1A methylation is associated with outcome. *BMC Cancer* **12** 243. (doi:10.1186/1471-2407-12-243)
- Yoshino A, Katayama Y, Ogino A, Watanabe T, Yachi K, Ohta T, Komine C, Yokoyama T & Fukushima T 2007 Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas. *Journal of Neuro-Oncology* **83** 153–162. (doi:10.1007/s11060-006-9316-9)
- Zhang X, Wen H & Shi X 2012 Lysine methylation: beyond histones. *Acta Biochimica et Biophysica Sinica* **44** 14–27. (doi:10.1093/abbs/gmr100)
- Zhu X, Lee K, Asa SL & Ezzat S 2007 Epigenetic silencing through DNA and histone methylation of fibroblast growth factor receptor 2 in neoplastic pituitary cells. *American Journal of Pathology* **170** 1618–1628. (doi:10.2353/ajpath.2007.061111)

Received in final form 3 June 2014

Accepted 4 June 2014

Made available online as an Accepted Preprint

4 June 2014