Epigenetics of thyroid cancer and novel therapeutic targets

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Abstract

An increasing body of evidence suggests that epigenetic changes (DNA methylation, remodeling and post-translational modification of chromatin) play important roles in thyroid tumorigenesis, as a result of their effects on tumor-cell differentiation and proliferation. Epigenetic silencing of various thyroid-specific genes has been detected in thyroid tumors. These changes can diminish the tumor’s ability to concentrate radioiodine, which dramatically reduces treatment options. Epigenetic changes in tumor-promoting and tumor-suppressor genes also contribute to the dysregulation of thyrocyte growth and other aspects of tumorigenesis, such as apoptosis, motility and invasiveness. We provide a brief overview of the mechanisms underlying epigenetic regulation of gene expression and the current methods used to investigate it. This is followed by a review of the principal epigenetic alterations detected in thyroid cancer cells, epigenetic strategies for treating thyroid cancers and data from preclinical and clinical studies (some still underway) on the use in this setting of demethylating agents and histone deacetylase inhibitors.

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Introduction

Progress in the field of thyroid cancer genetics has produced a novel class of drugs known as ‘targeted therapeutics’, which act selectively on cancer cells harboring particular genetic aberrations (Sherman 2010), and these agents are undergoing clinical testing for treatment of aggressive thyroid carcinomas (Schlumberger & Sherman 2009). The differentiation and proliferation properties of thyroid cancer cells are also strongly influenced by epigenetic alterations (Kondo et al. 2008), which are thought to be equally, if not more, important than mutational events in the generation and progression of human cancer (Feinberg et al. 2006, Chi et al. 2010, Taby & Issa 2010). Encouraging preliminary results have been obtained with epigenetic treatment strategies in several forms of cancer (Lane & Chabner 2009), and knowledge of the epigenetic changes that occur in thyroid carcinomas (often in combination with genetic alterations) is expected to reveal more effective ways to treat tumors of this type that fail to respond to currently available treatment modalities.

We provide a brief overview of the epigenetic alterations found in cancer cells in general and the methods currently available for their investigation. The main focal points of this review, however, are the roles played by epigenetic changes in controlling the differentiation and proliferation of transformed thyrocytes and the anticancer treatment strategies that target these changes.

Epigenetic gene regulation

Gene transcription depends strongly on chromatin structure: the open or loosely coiled conformation (euchromatin) has a permissive effect on transcription, whereas the closed conformation represented by tightly packed protein–DNA complexes (heterochromatin) is transcriptionally inactive. The conformational changes are based primarily on three mechanisms (Fig. 1).

The first is DNA methylation, which involves covalent attachment of a methyl group at position 5 in the cytosine ring that results in a methylcytosine. DNA methylation is a highly stable epigenetic modification (thus far, bona fide DNA demethylases have not been identified in any mammalian species), and it is considered the major obstacle to the reprogramming
Activation and arrest of gene transcription, respectively, acetylation and deacetylation, for example, cause epigenetic changes that can alter gene expression. Histone modifications seem to contribute significantly to the onset and progression of tumorigenesis, prompting a better understanding of the mechanisms involved.

Histone modifications include acetylation, methylation, ubiquitination, phosphorylation, and SUMOylation. These reactions are catalyzed by enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs). The transcriptional effects of these enzymes play roles in the development of cancer.

The third mechanism is post-translational modifications of the N-terminal tails of histones. These modifications include acetylation, methylation, ubiquitination, SUMOylation, and ADP ribosylation. These modifications can alter the dynamic competition between histones and transcription factors for cis-regulatory sequences in gene promoters, with important implications for cell differentiation and tumorigenesis.

Histone methylation is also important in tumorigenesis. Lysine and arginine residues in these proteins undergo mono-, di-, and tri-methylation catalyzed by histone methyltransferases (HMT), reactions that are reversed by histone demethylases such as KDM1/LSD1. The transcriptional effects of histone methylation depend on the residue involved and the number of methyl groups added. Agents like 3-deazaneplanocin that block HMT activity are emerging as promising anticancer compounds.

Early research on the epigenetics of cancer suggested that these molecular modifications were consequences of genetic mutations and relevant only to the progression (not the initiation) of tumorigenesis. The former concept has been challenged by the recent demonstration of a ‘field defect’ characterized by cancer cell-related epigenetic changes in the cells of normal peritumoral tissues. As for the latter view, several lines of evidence indicate that epigenetic changes are early events in the tumorigenesis process, prompting a better understanding of the mechanisms involved.
to propose an epigenetic progenitor origin of human cancer.

The investigation of epigenetic mechanisms in cancer has been greatly facilitated by the development of the chromatin immunoprecipitation (ChIP) procedure, which has recently been used to obtain interesting data on the epigenetic status of human thyroid cancer cells (Kondo et al. 2009).

ChIP uses specific antibodies to isolate and concentrate DNA sequences of interest (e.g. those containing methylcytosine or modified histones) from a sample containing a myriad of different proteins (Fig. 2). Single-gene readouts are obtained with quantitative PCR assay, but ChIP has also been successfully coupled with microarray (ChIP-chip) or massively parallel DNA sequencing (ChIP-seq) technology to generate genome-wide information (Gilchrist et al. 2009).

Applications in thyroid cancer

An important feature of the epigenetic effects on gene expression is their reversibility. In theory at least, cancer-promoting epigenetic alterations should be amenable to targeted drug therapy. Two potential epigenetic strategies have been identified for the treatment of thyroid carcinoma: the first involves the re-differentiation of tumors to restore their responsiveness to radioiodine therapy and the second entails the de-silencing of tumor-suppressor genes that can inhibit tumor cell growth and/or invasiveness.

Epigenetic control of differentiation

Thyroid cancer outcomes are strongly linked to the tumor’s avidity for radioiodine, which is used to detect and destroy residual, recurrent and metastatic cancer (Schlumberger et al. 2007). Many attempts have been made to re-differentiate transformed thyrocytes and restore their ability to concentrate the iodide so they can be treated with radioiodine, but thus far, the results have been of limited clinical value (Haugen 2004, Schlumberger et al. 2007, Seregni et al. 2009, Middendorp & Grünwald 2010). Radioiodine uptake and concentration by transformed thyrocytes require the same machinery used for thyroid hormone synthesis, a process controlled by TSH receptor (TSHR) signaling and mediated by thyroid-specific proteins (including thyroid peroxidase (TPO), thyroglobulin, the sodium/iodide symporter (NIS) and pendrin). As thyroid cancer advances, the genes encoding these proteins are frequently downregulated. The loss is progressive, beginning with the NIS and subsequently involving TPO, thyroglobulin and the TSHR itself (Lazar et al. 1999, Gerard et al. 2003; D Russo, G Damante, E Puxeddu, C Durante & S Filetti, Table 1 Clinical trials investigating the use of histone deacetylase inhibitors and demethylating agents in the treatment of radioiodine-refractory metastatic non-medullary thyroid carcinoma

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Testing phase</th>
<th>Status</th>
<th>Enrolled patients, n</th>
<th>Best response, n (%)</th>
<th>Complete response</th>
<th>Progressively growing disease, n</th>
<th>Progressive disease, n</th>
<th>Indeterminate, n</th>
<th>Status</th>
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<td>Vorinostat (SAHA)</td>
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<td>Phase I</td>
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<td>6</td>
<td>1 (20)</td>
<td>5 (83)</td>
<td>4 (67)</td>
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<td>recruiting</td>
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<tr>
<td>Romidepsin (^a)</td>
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<td>Phase II</td>
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References
in the thyroid-specific genes themselves (Shen & Chung 2005, Kon do et al. 2008). Preclinical studies indicate that both approaches are feasible and potentially effective. Suppression of signaling through the MAPK pathway (by knockdown of mutant BRAF or pharmacological inhibition of MEK) restores the expression of iodide-metabolizing genes in human thyroid carcinoma cells, highlighting this pathway as a critical target in re-differentiation strategies (Liu et al. 2007, Hou et al. 2010). On the epigenetic front, NIS and TSHR expression has been restored in human thyroid carcinoma cell lines with demethylating agents (Venkataraman et al. 1999, Xing et al. 2005, Tuncel et al. 2007). In poorly differentiated and undifferentiated thyroid carcinoma cells, HDACi can also reactivate the expression of NIS, TPO and thyroglobulin. In some cases, they also restore radiiodide uptake and retention (Kitazono et al. 2002, Zarnegar et al. 2002, Fortunati et al. 2004, Furuya et al. 2004, Puppin et al. 2005, Shen & Chung 2005, Shen et al. 2005a,b, Hou et al. 2010). The redifferentiating effects of some of these HDACi and demethylating agents are reportedly paralleled by inhibitory effects on cell proliferation (see next paragraph).

DNA methylation is dominant to histone deacetylation, so HDACi cannot reactivate transcription unless methylation has been inhibited. In in vitro studies, suppression of DNA methylation followed by inhibition of HDACs produced additive or synergistic effects in restoring the expression of transcriptionally silenced genes (Wischnewski et al. 2006). The advantages of this two-pronged re-differentiating approach to the treatment of cancer have been confirmed in in vitro studies (Li et al. 2007, Proenzano et al. 2007).

Epigenetic control of proliferation

Activation of tumor-promoting genes and inactivation of tumor suppressors – key events in thyroid tumorigenesis – are mediated by genetic and epigenetic modifications. The accumulation of epigenetic alterations is believed to contribute to the progressive dedifferentiation of thyroid cancers. The principal epigenetic modifications detected in these cancers are summarized in Table 2.

Thyroid cancer often exhibits downregulated expression of molecules (e.g. p27KIP1 and p16INK4A) that inhibit the cyclin-dependent kinases, thereby preventing the cell cycle from advancing into the S phase (Elisei et al. 1998, Khoo et al. 2002). In 30% of all thyroid tumors, CpG island methylation is detected in the p16INK4A gene promoter (Elisei et al. 1998). A similar percentage of thyroid tumors (benign tumors and papillary, follicular and anaplastic cancers) exhibit promoter methylation involving the RAS association family 1A (RASSF1A) tumor-suppressor

unpublished observations). The mechanisms underlying the dysregulation of iodine metabolism in thyroid cancer are still unclear. Presumably, genetic events result in a loss of physiological properties in neoplastic cells that parallels tumor dedifferentiation (Kondo et al. 2008). For example, the BRAF V600E mutation in papillary thyroid cancers is associated with reduced expression of key iodine-metabolism genes that is reflected in the tumors' clinical behavior (Durante et al. 2007, Puxeddu et al. 2008). Loss of NIS expression has also been documented in Ki-ras-transformed rat thyroid cells (Trapasso et al. 1999).

Several lines of evidence suggest that epigenetic mechanisms contribute to these changes. Promoter hypermethylation and silencing of the TSHR gene have been documented in thyroid carcinomas (Xing et al. 2003, Hoque et al. 2005), and similar findings have been reported for NIS and pendrin (Venkataraman et al. 1999, Neumann et al. 2004, Xing 2007). Furthermore, the expression of TTF1, a transcription factor that regulates the expression of all thyroid-specific genes (Bingle 1997), has been found to be silenced in poorly differentiated thyroid cancers as a result of promoter hypermethylation and histone H3 modifications (Kondo et al. 2009).

Redifferentiation involves the de-silencing of these iodine-metabolism genes (especially NIS and TSHR). It can be achieved by inhibiting molecular pathways activated by oncogene mutations or epigenetic changes

Figure 2  Schematic representation of chromatin immunoprecipitation-based approaches. Formaldehyde crosslink is required to covalently fix proteins to be immunoprecipitated and target DNA sequences. Omitting this step greatly reduces sensitivity of chromatin immunoprecipitation. Once protein and DNA have been immunoprecipitated, decrosslink is necessary to release target DNA sequences, which are analyzed in the subsequent steps. Crosslink and decrosslink are omitted in DNA methylation assays. For further details, see Collas (2010).
Table 2  Epigenetic modifications of tumor suppressors in thyroid cancer: hypermethylated genes and their prevalence

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Prevalence</th>
<th>References</th>
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<tbody>
<tr>
<td>p16INK4A</td>
<td>Cyclin dependent kinase inhibitor</td>
<td>30% of thyroid cancer</td>
<td>Elisei et al. (1998)</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>Microtubule stabilizer</td>
<td>30% of thyroid cancer</td>
<td>Xing et al. (2004) and Nakamura et al. (2005)</td>
</tr>
<tr>
<td>Rap1GAP</td>
<td>Rap1 GTPase-activating protein</td>
<td>72% of PTC</td>
<td>Zuo et al. (2010)</td>
</tr>
<tr>
<td>PTEN</td>
<td>Modulator of the PI3K/akt pathway</td>
<td>50% of PTC</td>
<td>Alvarez-Nuñes et al. (2006)</td>
</tr>
<tr>
<td>E-cadherin/CDH1</td>
<td>Regulator of cellular adhesion</td>
<td>Many CV-PTC 3 out of 5 DSV-PTC</td>
<td>Rocha et al. (2001)</td>
</tr>
<tr>
<td>hMLH1</td>
<td>DNA repair gene</td>
<td>38% PTC (correlated with BRAF mutation)</td>
<td>Guan et al. (2008)</td>
</tr>
<tr>
<td>SLC5A8</td>
<td>Sodium solute symporter family</td>
<td>33% PTC (associated with BRAF mutation)</td>
<td>Hu et al. (2006)</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>Tissue inhibitor of metalloproteinase</td>
<td>53% PTC (associated with BRAF mutation)</td>
<td>Hu et al. (2006)</td>
</tr>
<tr>
<td>DAPK</td>
<td>Ca/calmodulin-dependent ser/thr kinase protein</td>
<td>34% PTC (associated with BRAF mutation)</td>
<td>Hu et al. (2006)</td>
</tr>
<tr>
<td>RARß2</td>
<td>Negative regulator of cell growth</td>
<td>22% PTC (associated with BRAF mutation)</td>
<td>Hu et al. (2006)</td>
</tr>
</tbody>
</table>

PTC, papillary thyroid carcinoma; CV-PTC, classical variant-PTC; DSV-PTC, diffuse sclerosing variant-PTC; FTC, follicular thyroid carcinoma.


Rap1GAP is a Rap1 GTPase-activating protein that inhibits the RAS superfamily protein Rap1 by facilitating hydrolysis of GTP to GDP. In human thyroid cancers, Rap1GAP expression is frequently lost or downregulated secondary to promoter hypermethylation and/or loss of heterozygosity (Zuo et al. 2010). This loss correlates with tumor invasiveness but not with mutations known to activate MAPK signaling. Some investigators have suggested that Rap1GAP depletion promotes the progression of human thyroid tumors, possibly by allowing unrestrained Rap1 activity (Nellore et al. 2009). Interestingly, when thyroid cancer cell lines are treated with the demethylating agent 5-aza-deoxycytidine and/or the HDACi trichostatin A, Rap1GAP is re-expressed and inhibits proliferation (Zuo et al. 2010).

Nonexpression of another tumor-suppressor gene, phosphatase and tensin homologue (PTEN), has also been implicated in the development of thyroid cancer (and many other human cancers as well; Tell et al. 2004). The phosphatase it encodes dephosphorylates phosphatidylinositol-3,4,5-triphosphate and blocks signaling through the PI3K/AKT pathway (Cantley & Neel 1999). The frequency of PTEN silencing in thyroid tumors exceeds that of PTEN mutations or deletions. The detection of PTEN promoter hypermethylation in about 50% of papillary carcinomas and almost 100% of follicular carcinomas and adenomas suggests that it may be involved in thyroid tumorigenesis (Alvarez-Nuñes et al. 2006).

Activating BRAF mutations in papillary thyroid carcinomas have been linked to aberrant methylation of several tumor-suppressor genes, including TIMP3, SLC5A8, DAPK and RARß2 (Hu et al. 2006). Methylation of these genes correlated with signs of aggressive behavior in thyroid neoplasms, including extrathyroidal invasion, lymph node metastasis and advanced tumor stage at diagnosis, and their epigenetic silencing may be an important mechanism by which BRAF mutation promotes cancer progression (Xing 2007).

The epigenetic profiles of thyroid cancers (like their genetic counterparts) seem to vary widely. In series analyzed thus far, no single epigenetic change has ever been reported in 100% of the cases. However, none of the series reported has ever been comprehensively assessed for all known epigenetic alterations (or even the most important). A clearer picture of the frequencies of the various types of epigenetic changes found in thyroid cancer and pre-treatment knowledge of the specific changes harbored by single tumors might allow more effective therapeutic use of epigenetic modulators.

The observation that thyroid cancer progression is accompanied by an accumulation of epigenetic changes, many of which involve tumor-suppressor genes, has fueled attempts to reverse the malignant cell phenotype with demethylating agents and HDACi. In the few studies conducted with decitabine (alone or in combination with retinoic acid), the treatment suppressed the growth-inhibiting effects of HDACi on cancer cells (Miasaki et al. 2008, Vivaldi et al. 2009). The growth-inhibiting effects of HDACi on cancer cells have been more extensively investigated. Depsipeptide alone suppressed growth in a primary culture of metastatic BRAF-V600E papillary thyroid cancer cells, and synergistic effects were noted when it was used with paclitaxel and lovastatin or with gefitinib (Copland et al. 2011).
It has also been shown to sensitize a human anaplastic thyroid cancer (ATC) cell line to doxorubicin (Kitazono et al. 2002). Depsipeptide induces RhoB activity, which leads to upregulated p21 expression that attenuates cell proliferation (Marlow et al. 2009). Valproic acid strongly suppresses the growth of poorly differentiated thyroid cancer cell lines by inducing apoptosis and cell cycle arrest (Catalano et al. 2005, 2006). The HDACi suberoylanilide hydroxamic acid (SAHA), also known as vorinostat, reportedly induced growth arrest and caspase-mediated apoptosis in ATC cells. These effects were mediated by increased p21 protein levels, retinoblastoma protein hypophosphorylation, upregulated Bax expression and downregulated expression of Bcl-2 and Bcl-xL (Mitsiades et al. 2005). SAHA also suppressed the growth of ATC xenografts in mice (Luong et al. 2006). Its inhibitory effects on cancer growth have recently been ascribed to its repression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein degradation, which involves negative modulation of the ubiquitin-dependent pathway (Borbone et al. 2010). Even more promising results are being obtained with another HDACi, LBH589 (panobinostat), which affects the growth of undifferentiated thyroid cancer cells at nanomolar concentrations, reducing cell viability and triggering G2/M cell cycle arrest and apoptosis (Pugliese et al. 2009). LBH589’s action appears to be linked to microtubule stabilization (mediated by tubulin acetylation), upregulation of p21 expression and histone acetylation-mediated downregulation of cyclin D1 expression.

Concluding remarks and future directions

Preclinical studies have furnished convincing evidence that deacetylation inhibitors and demethylating agents are beneficial in the treatment of thyroid cancer, and these drugs are now being tested against metastatic radiodine-refractory thyroid carcinomas. Despite promising phase-I results (Kelly et al. 2005), however, SAHA treatment of 16 patients with differentiated thyroid carcinoma produced no partial or complete responses that met response evaluation criteria in solid tumors (Woyach et al. 2009), and in a phase-II study, i.v. injection of depsipeptide restored radiodine avidity in 2 of the 20 patients treated, but there were no objective responses even after 131I treatment (Sherman et al. 2009). In light of these preliminary data, epigenetic strategies seem far less promising than approaches that target protein kinases. Indeed, protein kinase antagonists have produced decidedly better response rates in clinical trials (although they are by no means free of adverse effects; Sherman 2010). However, the safety and efficacy of other HDACi and demethylating agents, alone or combined, are still being assessed (Table 1). Until the results of these trials become available, research on epigenetic alterations in thyroid cancer must continue with the ultimate objective of developing more effective treatments for 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 research reported.

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Epigenetics of thyroid cancer


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