Epigenomics in respiratory epithelium carcinogenesis: Prevention and therapeutic challenges

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Summary Respiratory epithelium carcinogenesis is currently considered as the phenotypic aspect of serial genetic and epigenetic aberrations resulting in deregulation of cellular homeostasis. Recent data indicate that DNA demethylating agents and histone deacetylase inhibitors might act synergistically for the prevention of cancer development throughout the carcinogen-exposed epithelium. Preliminary clinical trials have shown encouraging results using these new molecules in lung carcinomas therapeutics. However, the caveats that should be overtaken for efficacious antitumour activity have also emerged. Setting the context in which epigenetic modifications contribute to carcinogenesis evolution is of paramount importance in order to optimize the potency of the current and future epigenome targeting agents.

KEYWORDS
Lung cancer; Epigenomics; Methylation; Acetylation; Histone code

Epigenome and cancer

DNA is the foundation of life. Genetic derangements have been linked to various pathological entities, and they are considered as a hallmark of cancer. DNA is condensed into the nucleus in association with histones forming a complex of nucleosomes that comprise the structural subunit of coiled chromatin. The dynamic status of chromatin configuration (loose or tight) has profound influence on gene expression. Highly effective mechanisms have been identified that can modify in a temporal/spatial manner chromatin organization. Methylation status of cytosine residues in promoter-located clustered CpG islands and post-translational covalent histone modifications constitute heritable epigenetic changes affecting gene expression without altering DNA coding sequence.1

Despite their well-recognized key role during normal development, the implication of epigenomics in carcinogenesis is now considered determinative.2 As it is the case in most solid tumours, respiratory epithelium carcinogenesis is governed by the repression of tumour suppressor genes and/or activation of oncogenes.3 However, gene silencing

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is currently reassigned in a more sophisticated model that integrates both the “two-hit” hypothesis and epigenetic imprinting. The new epigenome era holds great promise for the prevention and/or treatment of respiratory epithelium carcinomas as epigenetic changes in tumour cells represent potentially reversible events, albeit several obscure points have to be enlightened.

Epigenomics during respiratory epithelium carcinogenesis

A gamut of histone post-translational modifications (acetylation, methylation, phosphorylation, ubiquitination) cooperatively affect transcriptional control of genes. Acetylation of lysine residues by histone acetyltransferases (HATs) results in chromatin decompression, formation of transcription co-factors-activating complexes, and orchestrated cooperation with DNA-binding gene-specific transcription factors and basal transcriptional machinery, thus providing specificity and plasticity. Mutations and chromosomal translocations of HATs, among them CREB-binding protein (CBP)/p300, p300/CBP-associated factor (PCAF), steroid receptor co-activator (SRC-1), and TATA box-binding protein-associated factor (TAF250) have been implicated in carcinogenesis. Although the role of CBP/p300 in haematologic malignancies is established, data suggest that it might also share cardinal role during respiratory epithelium carcinogenesis. The equilibrium of histone acetylation is dictated by the opposing actions of histone deacetylases (HDACs). Three major families of HDACs have been recognized and multiple mechanisms seem to engage them in cancer development. For instance, an abnormal increase of HDACs activity may lead to transcriptional inactivation of tumour suppressor genes [p53, retinoic acid receptor β (RARβ)], while others (Rb) require these enzymes to exert their function. However, the so-called “histone code” encompasses a wide range of mutually interacting combinations of histone modifications that modulate the transcriptional activity of chromatin. For example, histone methylation, in contrast to acetylation, can result in either transcription activation or repression, depending on the affected residue and other co-existing modifications.

Moreover, non-histone substrates, among them transcription factors (p53), chaperone proteins (Hsp90), and others (α-tubulin), of these biochemical processes are increasingly recognized as crucial coefficients of various carcinogenesis aspects. Inhibition of HDACs can reverse gene silencing in certain instances, but not in genes containing hypermethylated CpG islands.

The transfer of a methyl group to the C-5 position of cytosines, almost always in the context of CpG dinucleotides, is the most studied epigenetic event. DNA methylation requires two components, DNA methyltransferases (DNMTs) and methyl CpG-binding proteins (MBPs) (Fig. 1). Although the vast majority of normal cells genome is unmethylated, promoters of certain genes can undergo DNA methylation, causing transcriptional blockade. DNA methylation is intimately connected to respiratory epithelium carcinogenesis due to deregulation of DNMTs (enzymes involved in maintaining methylation patterns), regional gene hypermethylation (tumour suppressor genes, genes involved in cell-cycle control, apoptosis, and drug sensitivity), or through global hypomethylation that enhances oncogene expression and predisposes to genomic instability. Such aberrant DNA methylation occurs often in a tumour-specific fashion and has been correlated with dismal prognosis. Several genes have been shown to be hypermethylated in lung cancer patients’ specimens, fluids, and exfoliative material, as well as in cases of pre-malignant lesions and normal-appearing respiratory epithelium of high-risk individuals. Among them are genes that are involved in carcinogen activation or detoxification (CYP1A1, GSTP1), cell-cycle regulation (p16), DNA repair (MGMT), differentiation, (TCF21, RARβ), apoptosis (DAPK, caspase 8, Fas, TRAILR1), invasion and metastasis (cadherins, TIMP3, laminins, semaphorines, RECK), Ras/mitogen-activated protein kinase (MAPK) molecular pathway (RASSF1A, NORE1A), and others. Several techniques have been developed to analyze DNA methylation patterns, but the most applicable technique for large-scale prospective population studies has not yet been thoroughly validated. Most currently used assays for genome-wide methylation analysis rely on array-based hybridization, including methylation-specific restriction enzymes, methylation-specific protein binding and hybridization of bisulfite-treated DNA.

The mechanisms of epigenetic-mediated transcriptional repression are not yet fully understood. They involve direct interactions of methylated DNA with regulatory proteins, binding of MBPs, and recruitment of silencing complexes. DNA methylation and “histone code” consist a highly delicate intracellular cross-talking module. The hierarchical sequence of these events might represent a cellular/tissue-specific feature. Nevertheless, their disrupted cooperative functionality seems to be a prerequisite for respiratory epithelium carcinogenesis ignition and progression.

Prevention and therapeutic implications – a wave to the future

Genetic alterations that promote carcinogenesis are inherited passively through DNA replication. Epigenome changes are relatively frequent, affect multiple genes, are potentially reversible, occur since the early stages of respiratory epithelium carcinogenesis, and are established through various enzymatic activities, which can be theoretically targeted. Pharmacologic inhibition of these enzymes might restore the distorted epigenetic network and have therapeutic effect throughout the carcinogenesis process. A proof of principle of this assumption was the recent approval of 5-azacytadine for the treatment of myelodysplastic syndromes and suberylanilide hydroxamic acid (SAHA) for patients with progressive, persistent or recurrent forms of cutaneous T-cell lymphoma. So far, several inhibitors of DNMTs and HDACs are known to modify epigenetic information, but in a non-gene-specific manner. Furthermore, since multiple genes may be epigenetically deregulated during respiratory epithelium carcinogenesis, it still remains a question of which are the biologically most important ones that should be monitored and/or targeted.

Overexpression or abnormal recruitment of HDACs contributes to transcriptional “switch off” of pivotal genes during carcinogenesis. Currently, a number of different
classes of natural and synthetic HDACs inhibitors have been entered in the clinical testing field, such as hydroxamic acids (trichostatin A, SAHA, NVP-LAQ-824, PXD-101, CRA-024781), carboxylic acids (butanoic, valproic, 4-phenylbutanoic), benzamides (MS-275, N-acetyladinaline), epoxides (depeuducin, trapoxin A), and others (depsipeptide FK-228, apicidin).26,27 The most common outcome of HDACs inhibition is differentiation triggering, growth arrest, and/or apoptosis of tumour cells, although this refers to only 2–10% of target genes. Initial studies in lung cancer cell lines revealed that HDACs inhibitors modes of action are complex and the cross-relation with non-histone molecular pathways and DNA methylation nearly ‘chaotic’.28 HDACs inhibitors can also sensitize the antineoplastic effect of conventional cytotoxics and such combinations are being pursued, whilst the identification of surrogate markers of histone acetylation status is still pending.29

Figure 1 (a) Schematic representation of the serial phenotypic changes and the accumulated molecular alterations during respiratory epithelium carcinogenesis. Histologic evolution parallels the genetic events that occur at different time points, whereas the number of epigenetic deviations present in a lesion increases as the morphologic features worsen. (b) The two layers of epigenetic control, DNA methylation and ‘histone code’, are integrally cross-linked. Demethylating agents, histone deacetylase inhibitors and modulators of other key histone post-translational modifications may provide a means to achieve an optimal preventive and/or therapeutic outcome. Permutations of these approaches and continued advancement in understanding the mechanisms involved in epigenetic regulation and the way they interact with genetic changes during respiratory epithelium carcinogenesis, represent an upmost scientific niche. BTM, basal transcriptional machinery; Co-A; transcription co-activators; Co-R, transcription co-repressors; DNMTs, DNA methyltransferases; GSTFs, gene-specific transcription factors; HATs, histone acetyltransferases; HDACs, histone deacetylases; MBPs, methyl CpG-binding proteins.
could result in antitumour activity by either apoptosis induction, cellular differentiation enhancement, growth arrest or a combination of these cellular events. Cytosine methylation of CpG islands within the promoter regions of target genes results in transcriptional silencing. To date, DNA methylation targeting involves drugs that mainly hinder DNMTs, such as 5-azacytidine, 5,6-dihydroxy-azacytidine and 5-aza-2’-deoxycytidine (DAC) and, therefore, could theoretically reactivate the expression of such genes.\(^{30,31}\) (Fig. 1b). The optimal dosing scheme to sustain their epigenetic-related effect while minimizing their dose-limiting toxicities has not yet been determined. Other anti-DNMTs remedies, such as zebularine, hydralazine, procainamide, procaine, and (−)-epigallocatechin-3-gallate are also being clinically evaluated in lung cancer, although their preliminary results are not remarkable.\(^{32}\) Most currently known methylating agents act in a global manner. Therefore, they may also disrupt essential methylation or augment unwanted hypomethylation. This consideration is particularly relevant to the future perspectives of these agents in the chemoprevention field. However, their short clinical experience does not support these concerns, possibly because in normal cells transcriptional control by CpG promoter methylation is not a common and/or solitary event.\(^{32}\) Methylation patterns could be either incorporated in future screening trials\(^{33}\) or used as prognostic and/or predictive factors of various treatment modalities.\(^{34}\) However, it should be noted that methylation profile has profound heterogeneity among individual tumour types and even between separate histologic subtypes, the field-cancerization concept should always be considered in the case of respiratory epithelium carcinogenesis, whereas different carcinogens seem to preclude specific methylation changes.\(^{35}\) A broadly accepted definition of the normal methylation pattern is also necessary for the application of high-throughput techniques, because of the existence of inter-individual variability due to the presence of methylation polymorphisms in the human population.

Combined application of DNMTs and HDACs inhibitors represents a coherent approach and it has produced positive experimental results in controlling cancer cell proliferation, thus providing the impulse for combinatorial epigenome experimental results in controlling cancer cell proliferation, represents a coherent approach and it has produced positive techniques, because of the existence of inter-individual variation is also necessary for the application of high-throughput techniques, because of the existence of inter-individual variability due to the presence of methylation polymorphisms in the human population.

In conclusion, epigenome targeting in cancer therapeutics is no longer considered a scientific “chimera”. The intricate interplay of epigenetic circuitry is gradually unveiled, but its global grasp needs rational and daring innovations both in vitro and in clinical field. The time has come for a leap forward to a novel envisagement of respiratory epithelium carcinomas prevention and treatment.

References