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Epigenetics and Tumor Suppressor Genes

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1. Introduction

Genes which protect cells from malignant transformation were referred to as tumor suppressor genes (TSGs). Since the first description of TSG, *Rb* (retinoblastoma susceptibility gene), a myriad of genes have been identified as TSGs. These TSGs play critical roles in cell cycle control, apoptosis, DNA damage detection and repair, adhesion, metastasis, senescence, and carcinogen detoxification. Loss function of TSGs may cause uncontrolled cell growth and cancer. TSGs may be inactivated by different mechanisms during carcinogenesis. In addition to genetic changes, epigenetic aberration plays an important role in inactivation of TSGs. Epigenetics is described as heritable changes in gene expression that do not involve a change in the DNA sequence (Berger et al., 2009). DNA methylation and histone modification are two predominant epigenetic changes. More recently, non-coding RNAs were regarded as new epigenetic regulation tools. The purpose of this chapter is to describe the effects of epigenetic modification on TSGs.

2. Epigenetic changes during carcinogenesis

Initially, cancer was thought to be driven by a series of genetic changes. Epigenetics is now recognized as more important player in the initiation and progression of cancers (Rodríguez-Paredes & Esteller, 2011). DNA methylation at the cytosine residue of the CpG dinucleotides is one of the best-studied epigenetic changes (Bird, 2002; M.M. Suzuki & Bird, 2008). In normal cells, CpG loci are methylated scatteringly across the genome. By contrast, short CpG-rich DNA regions, called 'CpG islands', are normally unmethylated. These 'CpG islands' are preferentially located in the promoter region of about 60% of human genes. Global DNA hypomethylation was the first epigenetic alteration found in human cancer (Feinberg & Vogelstein, 1983). Hypomethylation may lead to deleterious consequences, including genome instability, activation of transposable elements, or loss of genomic imprinting (Esteller, 2008). However, promoter-specific hypermethylation was regarded as the major epigenetic change of cancer, which is associated with TSGs silencing (Herman & Baylin, 2003).

Histone modification is another kind of epigenetic changes. Histones are subject to a wide range of post-transcriptional modifications in their N-terminal tails, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and ADP-ribosylation (Kouzarides, 2007; Campos & Reinberg, 2009). It has been proposed that distinct histone modifications form different 'histone codes' (Strahl & Allis, 2000). Generally, histone

acetylation is associated with transcriptional activation, while the role of histone methylation in gene expression relies on the specific residue and methylation state. One of the common hallmarks of human cancer is global loss of monoacetylation of lysine (K) 16 and trimethylation of lysine 20 on histone H4 (H4K16ac and H4K20me3) along with hypomethylation in repetitive DNA sequences (Fraga et al., 2005). Conversely, loss of acetylation of H3K9 and H4K16 (H3K9ac and H4K16ac) as well as trimethylation of H3K4 (H3K4me3) and gain of trimethylation of H3K27 (H3K27me3) and dimethylation of H3K9 (H3K9me2) occur at the promoters of TSGs and contribute to tumorigenesis by silencing of these critical genes (Figure 1) (Esteller, 2007a). In brief, aberrant 'epigenomes' marked by global DNA hypomethylation, promoter-specific hypermethylation, and abnormal histone modifications are main epigenetic changes in cancer. Since silencing of TSGs caused by CpG island hypermethylation and repressive histone modification is the common epigenetic event in human cancers, the following discussion will focus on the epigenetic silencing of TSGs during tumorigenesis.

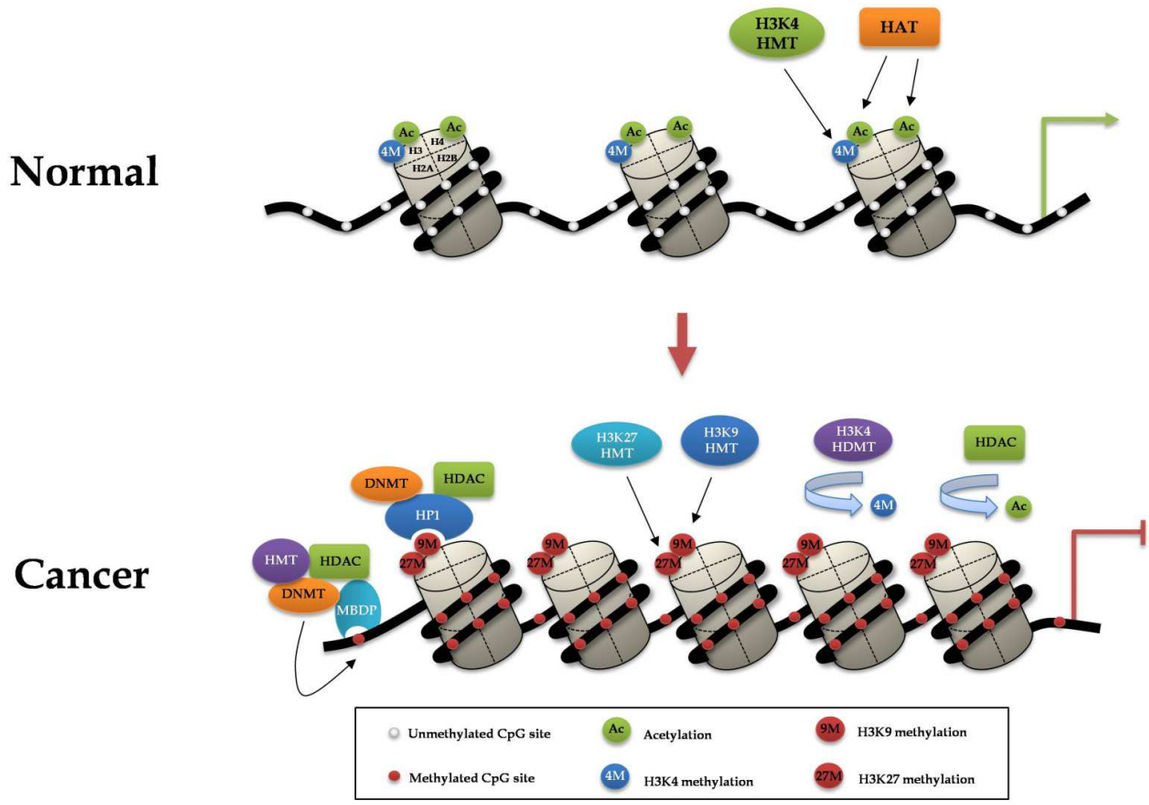

Fig. 1. Mechanisms of TSGs silencing by epigenetic changes during carcinogenesis.

In normal cells, promoter region is unmethylated and possesses active histone modifications (e.g., H3K4me and acetylation of H3 and H4). Transcription of TSGs was activated. In cancer cells, the promoter region is densely methylated, active histone modifications were lost and inactive histone modifications were induced (e.g., hypoacetylation of histones H3 and H4, loss of H3K4me3, and gain of H3K9me and H3K27me3). MBDPs bind to methylated DNA. HDACs and HMTs were recruited. Transcription of TSGs was inactivated.

3. DNA methylation of TSGs

3.1 DNA methyltransferase

DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which add methyl groups to the cytosine of CpG dinucleotides. Three main DNMTs have been identified. DNMT1 maintains the existing methylation patterns following DNA replication, whereas DNMT3A and DNMT3B are responsible for *de novo* methylation patterns (Bird, 2002; M.M. Suzuki & Bird, 2008). Overexpresion of DNMTs has been observed in cancers, which contributes to CpG island hypermethylation of TSGs and concomitant silencing of gene expression (Robert et al., 2002; Nosho et al., 2009). Although DNMTs have been classified as maintenance or *de novo* methyltransferases, all three DNMTs participate in both *de novo* and maintenance methylation, and cooperate to silence TSGs in human cancer (Rhee et al., 2000; G.D. Kim et al., 2002; Rhee et al., 2002). More recently, three independent groups revealed that somatic mutations in DNMT3A occur in acute myeloid leukemia (AML), and lead to some gene expression and methylation changes (Shah & Licht, 2011). The other DNMTs, including DNMT3L and DNMT2, were reported recently. DNMT3L appears to be required for the methylation of imprinted genes in germ cells, and interacts with DNMT3a and 3b in *de novo* methyltransferase activity (Chen et al., 2005). But the biological function of DNMT2 remains unclear, its strong binding to DNA suggests that it may mark specific sequences in the genome (Dong et al., 2001).

3.2 Hypermethylation of TSGs in cancer

Promoter region hypermethylation is accepted as the mechanism of inactivation of TSGs in human cancers. The initial finding of CpG island hypermethylation of *Rb* in human cancer (Greger et al., 1989) was followed by the discovery of other TSGs undergoing methylation-associated inactivation, such as *VHL* (von Hippel-Lindau tumor suppressor), *p16INK4a* (cyclin-dependent kinase inhibitor 2A [CDKN2A]), *BRCA1* (breast-cancer susceptibility gene 1), and *hMLH1* (mutL homolog-1) (Esteller, 2002, 2008). These methylated TSGs are distributed in all cellular pathways relevant to tumor development, such as cell cycle regulation, DNA repair, apoptosis, transcriptional regulation, carcinogen-metabolism and drug resistance, angiogenesis, metastasis and cell-adherence (Esteller, 2002, 2008).

Hypermethylation of TSGs occurs at any time during carcinogenesis, especially in the early stages of the neoplastic process, which may facilitate cells to obtain further genetic lesions (Feinberg et al., 2006). One example is hypermethylation of DNA repair gene *MGMT* (O6-methylguanine-DNA methyltransferase) in the early phase of tumorigenesis, which results in the accumulation of genetic mutations that arise from the defects in DNA repair (Esteller et al., 2001a; Kuester et al., 2009). In addition, silencing of TSGs by promoter hypermethylation also let neoplastic cells addict to a particular oncogenic pathway, such as loss of *SFRP* (secreted frizzled-related proteins) expression in early stage of colon cancer activating the Wnt pathway (Baylin & Ohm, 2006). Furthermore, hypermethylation-induced silencing of transcription factors, such as *GATA-4* and *GATA-5* in colorectal and gastric cancers (Akiyama et al., 2003) as well as in esophageal cancer (Guo et al., 2006a), can also lead to inactivation of their downstream targets. Importantly, the increasing atypia observed at the histologic level is associated with the increasing number of methylated CpG islands at gene promoter regions. Our previous study suggested that the accumulation of DNA methylation was happened during esophagus carcinogenesis (Guo et al., 2006b).

The patterns of aberrant methylation of TSGs may represent different tumor types (Costello et al., 2000; Paz et al., 2003). Hypermethytion of *GSTP1* (glutathione S-transferase-π) was found in 80–90% of prostate cancers but hardly in other tumor types (Lee et al., 1994; Esteller et al., 1998; Cairns et al., 2001). Another finding indicated that *CDX2* (caudal related homeobox gene) methylation is a feature of squamous esophageal cancer (Guo et al., 2007). Tumor-type specific hypermethylation occurs not only in sporadic tumor but also in inherited cancer syndromes (Esteller et al., 2001b), where hypermethylation serves as the second hit in the Knudson's two-hit model for TSG inactivation (Grady et al., 2000). But some TSGs, such as *BRCA2*, *hMSH2*, *hMSH3*, *hMSH6*, *p19INK4d*, *CHK1*, *CHK2*, *MTAP* and *NKX3.1*, are rarely methylated in caner (Esteller, 2007b). The mechanism of tumor-type specific methylation remains unclear. Several hypotheses have been proposed to explain this phenomenon: (1) in certain tumor type hypermethylation might occur at particular genes which confer a selective clonal advantage; (2) there are common sequence motifs in the hypermethylated promoters of TSGs (Esteller, 2007b); (3) selective DNA methylation can be directed by other chromatin players, such as Polycomb proteins, pinpointing 'methylable' islands (Schlesinger et al., 2006; Esteller, 2007b).

3.3 Mechanisms of TSGs silencing by DNA methylation

It was proposed as one of the mechanisms that DNA methylation may directly block the specific binding sites of transcription factors (Comb & Goodman, 1990; Deng et al., 2001). Another more acceptable mechanism is that methyl-CpG-binding proteins (MBDPs) recognize m5CpG sequences and silence transcription. There are five well-known MBDPs which were regarded as important "translators" between DNA methylation and transcriptional silencing, including MeCP2, MBD1, MBD2, MBD3 and MBD4 (Lopez-Serra & Esteller, 2008). MBDPs bind to methylated DNA, and then histone modification enzymes were recruited to establish silenced chromatin model (Nan et al., 1998; Fuks et al., 2003).

4. Regulation of TSGs by histone modifications

Hypermethylation of TSGs in human cancer was extensively studied. But limited researches were performed on the regulation of gene expression by histone modifications. One of the main reasons is lacking rapid and comprehensive methods to analyze the histone modifications (Esteller, 2007a; Taby & Issa, 2010). Importantly, the effective histone modifications were discovered during the past decade, especially histone acetylation and methylation on TSGs regulation.

4.1 Histone acetylation

Histone acetylation occurs mainly at lysine residues of the H3 and H4, and makes RNA polymerase and transcription factors easier to access the promoter region. Therefore, in general, the acetylation of histone lysines is associated with euchromatin and transcriptional activation of gene expression, whereas the deacetylated residues are associated with heterochromatin and transcriptional gene silencing. Histone acetyltransferases (HATs) and deacetylases (HDACs) are, respectively, responsible for the addition and removal of acetyl groups from lysine residues. The precise balance between HATs and HDACs determines the status of histone acetylation (Ellis et al., 2009; Taby & Issa, 2010). In cancer cells, disruption of the balance between HATs and HDACs contributes to transcriptional inactivation of

TSGs. The typical example of gene silencing by this mechanism is the inactivation of cyclin-dependent kinase inhibitor *p21WAF1* by hypoacetylation in the absence of CpG-island hypermethylation (Richon et al., 2000). Interestingly, some TSGs with CpG island hypermethylation, can also be re-expressed through inhibition of SIRT1 (a class III HDAC), which increases H4K16 and H3K9 acetylation at promoters without affecting the hypermethylation status (Pruitt et al., 2006). Furthermore, in addition to regulation of TSGs at transcriptional level, HATs/HDACs influence the activity of TSGs by post-translational modifications (Glozak et al., 2005). For example, p53 is subjected to extensive acetylation mediated by HATs such as Tip60 (Sykes et al., 2006) and p300 (Gu & Roeder, 1997) and can be deacetylated by HDACs like SIRT1 (Yi & Luo, 2010). The aberrant histone acetylation of TSGs during carcinogenesis may result from the alteration in HATs/HDACs. Inactivation of HAT activity through gene mutation (e.g., missense mutations of p300) or viral oncoproteins (e.g., the inactivation of p300 by E1A and SV40) has been reported in both hematological and solid tumors, whereas misdirection of HAT activities as a result of chromosomal translocations (e.g., mixed lineage leukemia protein [MLL]-CBP [MLL-CBP]) has been implicated in the onset and progression of acute leukemia (Ellis et al., 2009). On the other hand, overexpression of HDACs in solid tumors (Song et al., 2005) and aberrant recruitment them to specific promoters through interaction with proto-oncogenes in leukemias (Ellis et al., 2009) have also been reported.

4.2 Histone methylation

Similar to histone acetylation, histone methylation is dynamically regulated by the opposing activities of histone methyltransferases (HMTs) and histone demethylases (HDMTs), such as KDM1/LSD1 and the Jumonji domain-containing protein (JMJD) family. Methylation takes place on both lysine and arginine residues, and has different degrees, known as mono-, di-, and tri-methylation. In most instances, methylation at H3K9, H3K27 and H3K20 is associated with transcriptional repression, whereas methylation of H3K4, H3K36 and H3K79 is associated with transcriptional activation (Ellis et al., 2009; Taby & Issa, 2010). The shifting of balance between HMTs and HDMTs in cancer also causes the silencing of TSGs. For instance, the H3K27me3-specific HMT EZH2 (enhancer of zeste homolog 2), catalytic subunit of PRC2 (Polycomb-repressive complex 2), is overexpressed in a broad range of hematopoietic and solid tumors, including prostate, breast, colon, skin and lung cancer (Tsang & Cheng, 2011). Mechanistically, the overabundance of EZH2 in cancer leads to transcriptional silencing of TSGs, such as *RUNX3* and *DAB2IP* through trimethylation of H3H27 (Fujii et al., 2008; Min et al., 2010). Conversely, the H3K27me3 repressive mark is demethylated by UTX/JMJD3 proteins, which belongs to JMJD family (Agger et al., 2007). Loss-of-function mutations of UTX in human cancers suggest UTX as a tumor suppressor gene (Van Haaften et al., 2009). This mutation could increase H3K27me3 level, and inactive *Rb* (Herz et al., 2010; J.K. Wang et al., 2010). The altered expression profiles of other histone methylation-modifying enzymes or abnormal targeting of these enzymes also contribute to inactivation of TSGs, such as downregulation of *BRCA1* in breast cancer cells caused by overexpression of PLU-1 (a member of JMJD family responsible for demethylation of H3K4) (Yamane et al., 2007). Finally, it is worth to be mentioned that the histone methylation-modifying enzymes also directly target non-histone proteins (Lan & Shi, 2009). Similar to the case of acetylation, p53 activity can be regulated by methylation or demethylation through HMTs or HDMTs (Huang et al., 2007, 2010).

5. Regulation of TSGs by interplay between DNA methylation and histone modifications

In addition to the independent effect, DNA methylation and histone modifications may interact with each other to reorganize chromatin structure and gene expression (Cedar & Bergman, 2009; Murr, 2010). Promoter region hypermethylation of TSGs is associated with histone modifications in cancer cells (e.g., hypoacetylation of histone H3 and H4, loss of H3K4me3, and gain of H3K9me and H3K27me3) (Esteller, 2008) (Figure 1). These connections might be carried out by the direct interaction of DNA methylation machinery and histone modification enzymes (Cedar & Bergman, 2009). However, the question of which epigenetic change is the initial event still remains controversial. Emerging evidence indicates that histone modifications may induce DNA methylation. For example, H3K9me2 may be necessary for DNA methylation in some TSGs, such as *p16INK4a* (Bachman et al., 2003). In this model, H3K9me2 can serve as a binding site for heterochromatin protein 1 (HP1), and thus generating a local heterochromatin by interacting with DNMTs and HDACs (Smallwood et al., 2007). On the other hand, DNA methylation machinery may recruit histone modification enzymes as well. The dynamic epigenetic silencing of *GSTP1* in prostate cancers is one of the good examples. It was reported that CpG island methylation of *GSTP1* played a critical role in deacetylation of H3K9 and concomitant methylation of H3K9 (Stirzaker et al., 2004). The link of DNA methylation and histone modifications might be mediated by MBDPs, which could recruit the HDACs and HMTs to the promoter methylated target genes (Nan et al., 1998; Fuks et al., 2003; Stirzaker et al., 2004). Furthermore, DNMTs themselves are associated with histone modification enzymes, such as HDACs (Fuks et al., 2000), and G9a (Estève et al., 2006).

6. Regulation of epigenetic modification machinery by TSGs

The roles of epigenetic modifications in regulation of TSGs expression are widely accepted. As transcription factors, some TSGs may be involved in regulation of the epigenetic modification machinery. p53, one of the most well-documented TSGs, has been reported to regulate histone modification. HATs, such as p300/CBP and TRRAP, are recruited to target gene depended on binding of p53 to promoter, and thus induces gene expression (Barlev et al., 2001; Vrba et al., 2008). At the same time, p53 may cause repression of a subset target genes, such as *MAP4*, *AFP* and *Nanog* through recruiting SIN3A-HDAC (Murphy et al., 1999; Lin et al., 2004; Nguyen et al., 2005). More recently, Zeng et al showed that p53 recruit both HDAC and PcG to *ARF* locus to repress its expression by a negative feedback manner during normal cell growth (Zeng et al., 2011). Similar example was reported in RB protein. RB-mediated transcriptional repression was induced through the association with a variety of chromatin modification and remodeling enzymes, including DNMTs, HDACs, HMTs (Luo et al., 1998; Robertson et al., 2000; Kotake et al., 2007) and Brg1/Brm (Dunaief et al., 1994; Strober et al., 1996). The other examples, such as maspin was also known to direct epigenetic regulation. Maspin was regarded as an endogenous inhibitor of HDAC1 (Li et al., 2006). It is noticeable that the interaction of TSGs and histone modification enzymes may produce different outcomes. TSGs and histone modification enzymes may regulate each other, which may be determined upon different cellar states.

7. Non-coding RNAs enter epigenetic world

Non-coding RNAs (ncRNAs) are functional RNA molecules that do not code for proteins. Based on size, they are divided into different classes: long ncRNAs (lncRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), microRNAs (miRNAs), etc (Brosnan & Voinnet, 2009). NcRNAs can regulate gene expression through a diversity of mechanisms. Recently, a handful of studies have implicated ncRNAs in a variety of disease states, especially in cancer. Many ncRNAs, such as miRNAs and lncRNAs could play the similar roles as TSGs, and also function as oncogens that in turn regulate the expressions of TSGs in transcriptional and post-transcriptional level.

7.1 Interplay between MiRNAs and epigenetic machinery

MiRNAs are small ncRNAs with 19~22nt, which regulate gene expression via translational inhibition or mRNA degradation in a sequence-specific manner. MiRNAs could function as TSGs or oncogenes in cancer. In the last few years, increasing evidence has indicated that a substantial number of miRNA genes with tumor suppression functions are associated with CpG islands and silenced by epigenetic alterations in cancers. Indeed, miR-127 was found to be embedded in a CpG island region and epigenetically silenced by both promoter hypermethylation and histone modifications in cancer cells, and could be reactivated following treatment with combination of DNA demethylating agent and HDAC inhibitor (Saito et al., 2006). miR-9-1 was also found to be hypermethylated and consequently down-regulated in breast cancer (Lehmann et al., 2008) as well as the hypermethylation of clustered miR-34b and miR-34c in colon cancer (Toyota et al., 2008). Intriguingly, miRNAs are not only epigenetically regulated but also act as chromatin modifiers to regulate the gene expression (Valeri et al., 2009). Fabbri et al reported the first evidence that miR-29s (miR-29a, -29b, -29c) directly target DNMT3a and DNMT3b (Fabbri et al., 2007). After miR-29s treatment, the epigenetically silenced TSGs like *p15INK4b* and *ESR1* were re-expressed comparably to use of DNMT inhibitors (Fabbri et al., 2007; Garzon et al., 2009). Similarly, HMTs are also targets of miRNAs. Studies have shown that miR-101 exerts its tumor suppressive properties by targeting the EZH2 (Varambally et al., 2008; Friedman et al., 2009).

7.2 LncRNA: A new player in epigenetics

LncRNAs are emerging as new players in human cancers with potential roles in both oncogenic and tumor suppressive pathways, and the most fascinating thing is that they could play crucial roles in epigenetic modifications. Notably, evidence has suggested that lncRNAs can mediate epigenetic changes by recruiting chromatin remodeling complexes to specific genomic loci (Mercer et al., 2009). For example, *ANRIL*, a antisense to the *INK4n/ARF/INK4a* promoter, interacts with PRC1 component CBX7 to repress the transcription of *INK4n/ARF/INK4a* locus (Yap et al., 2010). On the other hand, lncRNAs could function as TSGs and modulate the epigenetic machinery by interaction with other proteins. In response to DNA damage, ncRNAs transcribed from the 5′ regulatory region of *CCND1*, binds to and activate TLS, which inhibits CBP/p300 histone acetyltransferase activities leading to repression of *CCND1* transcription (X. Wang et al., 2008).

8. Screening candidate TSGs by epigenetic strategies

TSGs are generally silenced by CpG island hypermethylation and repressive histone modifications. So, epigenetic signatures may be applied to screen tumor suppressor. It is

important to isolate epigenetically silenced genes in cancer. To this end, many procedures were reported. For example, by comparation of genes expression level before and after 5-aza-2'-deoxycytidine (5-aza-CdR) treatment, Suzuki et al isolated hypermethylation silenced genes *SFRPs* in colonic cancer cell lines and further analyzed their tumor suppressor function (H. Suzuki et al., 2002). Similarly, Gery et al employed microarray analysis to identify genes reactivated in lung cancer after combined treatment with 5-aza-CdR and SAHA. In this screen, *Per1* was identified as a candidate tumor suppressor in lung cancer, and DNA hypermethylation and histone H3 acetylation are potential mechanisms for silencing *Per1* (Gery et al., 2007). For the promoter CpG island hypermathylation detection, anti-mC immunological techniques, HPLC-TLC, HPCE, ERMA, bisulphite sequencing, MSP, MSP-ISH and DNA methylation mircroarray were employed (Laird, 2003). ChIP, ChIP coupled with microarray hybridization (ChIP-chip), ChIP coupled with next-generation DNA sequencing (ChIP-seq), mass spectrometry (Rasoulpour et al., 2011) were used to determine the regional or global repressive histone modifications (deacetylation of specific H3 and H4 lysine or methylation of H4K9/27 even the combination).

9. Clinical application

Understanding of how epigenetic alterations contribute to TSGs regulation would facilitate its transformation and clinical application. Based on the characters of stability, variability and reversibility, epigenetic modifications have potentials as both cancer biomarkers for detection, prognosis, and therapy prediction, and drug targets for cancer therapy (Mulero-Navarro & Esteller, 2008).

9.1 Epigenetic biomarkers

As described previously, each tumor type may be represented by a different methylation pattern. Promoter region Hypermethylation usually occurred in the early stage of carcinogenesis. Therefore it is possible to detect early lesions by examination of TSGs methylation. Previous study has shown that *HIN-1* (high in normal-1) methylation is an early event of human esophageal cancer (Guo et al., 2008). TSGs methylation can also be the predictors of tumor prognosis. For example, methylation of the promoter region of *p16INK4a*, *CDH13* (H-cadherin gene), *RASSF1A* (Ras association domain family 1 gene) and *APC* (adenomatous polyposis coli gene) in patients with stage I NSCLC treated with surgery is associated with increased risk of early recurrence (Brock et al., 2008). In addition, DNA methylation may serve as chemotherapy predictor. The representative methylation markers to predict drug-responsiveness are *MGMT* (Esteller et al., 2000), *hMLH1* (Plumb et al., 2000), *WRN* (the Werner syndrome–associated gene) (Agrelo et al., 2006), *IGFBP-3* (insulin-like growth factor–binding protein-3) (Ibanez et al., 2010), or *BRCA1* (Veeck et al., 2010) (Table 1).

9.2 Epigenetic agents

Unlike genetic mutations, epigenetic silenced TSGs can be awakened by drugs. Many epigenetic drugs have been discovered to rescue the functions of TSGs by reversing aberrant epigenetic changes. US Food and Drug Administration (FDA) have approved four epigenetic drugs for cancer therapy. Two DNMT inhibitors, 5-aza-CR (vidaza) and 5-aza-CdR (decitabine), were used in the treatment of myelodysplastic syndromes and leukemia, while two HDAC inhibitors, vorinostat (suberoylanilide hydroxamic acid [SAHA]) and romidepsin (FK-228), were applied in cutaneous T cell lymphoma (Rodríguez-Paredes &

Esteller, 2011). These drugs can be administrated in combination or independent manner. Despite promising results, epigenetic related therapy still remains challenge. Similar with epigenetic changes in TSGs, ncRNAs pattern in cancer may serve as diagnosis, prognosis and chemosensitivity marker and therapeutic target.

Hypermethylated TSGs	Gene Function	Representative Cancer Type	Ref.	Potential Clinical Application
GSTP1	Conjugation to glutathione	Prostate cancer	(Lee et al., 1994)	Detection
GATA-4/-5	Transcription factor	esophageal cancer	(Guo et al., 2006a)	
APC	Wnt signaling	Colorectal cancer; breast cancer	(Mulero-Navarro & Esteller, 2008)	
CDX2	Homeobox transcription factor	Squamous esophageal cancer	(Guo et al., 2007)	
p16INK4a	Cyclin-dependent kinase inhibitor	Colorectal cancer	(Esteller et al., 2001c)	Prognosis
SFRP1	Antagonists of Wnt signaling	Breast cancer	(Veeck et al., 2006)	
DAPK	Pro-apoptotic	NSCLC	(Tang et al., 2000)	
EMP3	myelin-related gene	glioma and neuroblastoma	(Alaminos et al., 2005)	
CDH1	E cadherin, cell adhesion	NSCLC	(D. S. Kim et al., 2007)	
CDH13	H cadherin, cell adhesion	NSCLC	(D. S. Kim et al., 2007)	
MGMT	DNA repair of 06–alkyl-guanine	gliomas	(Esteller et al., 2000)	Chemosensitivity
hMLH1	DNA mismatch repair	Ovarian and colon cancer	(Plumb et al., 2000)	
BRCA1	DNA repair, transcription	Breast cancers	(Veeck et al., 2010)	
WRN	DNA repair	Colorectal cancer	(Agrelo et al., 2006)	
IGFBP-3	Growth-factor-binding protein	NSCLC	(Ibanez et al., 2010	

CDH1 (E cadherin), EMP3 (epithelial membrane protein 3), DAPK (death-associated protein kinase).

Table 1. Representative epigenetic markers in cancer.

10. Conclusion

Aberrant epigenetic changes play important roles in human carcinogenesis. Major epigenetic changes include DNA methylation, aberrant histone modification and alterations of noncoding RNA patterns. The expression of TSGs was regulated by epigenetic modification. Epigenetic silencing of TSGs by promoter region hypermethylation in combination with repressive histone modifications was recognized as a common feature of various human cancers. Undoubtedly, understanding of the inactivation of TSGs is of fundamental importance in exploration of the pathogenesis and progression of cancer, and thus facilitating to yield attractive cancer biomarkers and therapeutic targets. The pivotal roles of ncRNAs in the development of cancer have refreshed the complicated epigenetic network, which provides a possibility on developing ncRNAs mediated diagnostics, prognostics and therapeutics. It is possible, in the near future, to find novel cancer-specific biomarkers and gene-specific drugs with low cytotoxicity.

11. References

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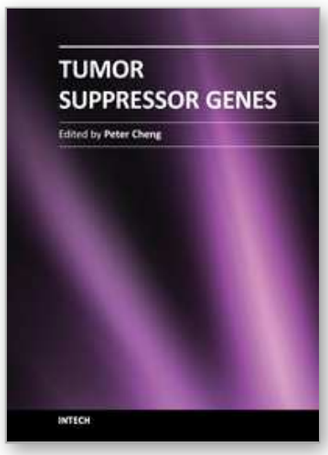

Tumor Suppressor Genes

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Functional evidence obtained from somatic cell fusion studies indicated that a group of genes from normal cells might replace or correct a defective function of cancer cells. Tumorigenesis that could be initiated by two mutations was established by the analysis of hereditary retinoblastoma, which led to the eventual cloning of RB1 gene. The two-hit hypothesis helped isolate many tumor suppressor genes (TSG) since then. More recently, the roles of haploinsufficiency, epigenetic control, and gene dosage effects in some TSGs, such as P53, P16 and PTEN, have been studied extensively. It is now widely recognized that deregulation of growth control is one of the major hallmarks of cancer biological capabilities, and TSGs play critical roles in many cellular activities through signaling transduction networks. This book is an excellent review of current understanding of TSGs, and indicates that the accumulated TSG knowledge has opened a new frontier for cancer therapies.

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