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# Recent advances in DNA methylation in tumorigenesis and diagnosis

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## Abstract

DNA methylation is the process of adding a methyl group to the 5'-carbon of the cytosine residue in the CpG dinucleotide sequence, and it is one of the key components of epigenetic modifications. DNA methylation occurs alongside various biological processes. Abnormal DNA methylation is often associated with the onset of various severe diseases. As a century-old problem that threatens human life, in-depth studies of tumors have revealed abundant evidence of dysregulated DNA methylation. Numerous studies have indicated that DNA hypermethylation tends to impact the transcription of many tumor suppressor genes, leading to the immortalization of tumor cells. In this review, we systematically summarize the progression of DNA methylation in tumor types with current high incidence rates. We also summarize the current clinical methods of DNA methylation detection and treatment and provide an in-depth analysis of the advantages and limitations of these methods. In addition, we discuss the future limitations and challenges faced by DNA methylation research, aiming to advance its clinical application in tumor diagnosis and treatment.

**Keywords:** Diagnosis, DNA methylation, Epigenetics, Treatment, Tumorigenesis

## 1. Introduction

Epigenetics is a discipline that reveals how life activities achieve heritable changes through modifications of the genomic sequence[1]. This process does not result in changes in the genome sequence, but brings about dynamic regulation of life activities, such as regulating biological growth[2], disease[3,4], and death. DNA methylation, as a form of epigenetic modification, has captured the attention of numerous researchers. During genome transcription, functional group modifications of DNA sequences exert different roles in cellular activities, such as the activation and deactivation of cis-acting elements and the stabilization of mRNA. Specifically, DNA methylation performs a crucial role in embryonic development, imprinting, and X-chromosome inactivation[5–7]. The DNA methylation process requires specific enzymatic reactions, namely DNA methyltransferases (DNMTs). These enzymes transfer the methyl group from S-adenosylmethionine (SAM) to the cytosine bases in CpG dinucleotides within promoter and regulatory regions[8]. CpG dinucleotides are concentrated in short CpG islands (CGIs), commonly referred to as "CGIs." In humans, CGIs account for approximately 60% of gene promoters, which will undergo methylation during the development of life, leading to long-term gene silencing. A typical example of naturally occurring CGI methylation is X-chromosome inactivation and imprinted genes[9,10]. Unfortunately, DNA methylation has been widely reported to be associated with the silencing of tumor suppressor genes and differentiation genes in various cancers[11].

In 1925, DNA methylation was initially identified in bacteria. Over the subsequent decades, the biological significance of DNA methylation was not given significant attention by researchers[10]. It was not until the advancement of high-throughput sequencing technologies that a deeper understanding of the regulatory mechanisms of DNA methylation was established[12]. As research into DNA methylation has been progressing, it is involved in various biological processes, ranging from gene regulation to cell growth[13], and the mechanisms underlying major diseases[14,15]. DNA methylation regulates gene expression by interacting with cis-acting elements. In normal cells, DNA methylation maintains genomic stability by suppressing the transcription of transposons and other repetitive elements. The dynamic regulatory mechanisms of DNA methylation are also crucial for maintaining normal stem cells and progenitor cells[16,17]. Analysis of embryonic stem cells has revealed that even in embryonic stem cells completely lacking DNA methylation, over 98% of methylated genes remained inactive, suggesting the presence of a potential mechanism that helps maintain their silence in somatic cells[18]. This mechanism appears to utilize SAM as a methyl donor, carried out by 3 DNMTs: DNMT1, DNMT3a, and DNMT3B[19,20]. DNA methylation patterns are initially established by DNMTs DNMT3A and DNMT3B and are subsequently maintained by DNMT DNMT1 during DNA replication. In tumors, dysregulation of DNMT activity increases the likelihood of genomic instability, thereby conferring the ability for unlimited proliferation to cells[21–23]. Of note, as it is a problem at the genetic level, DNA methylation brings about many challenges in the process of cancer treatment.

In this review, we provide an in-depth summary of the role of DNA methylation at different stages of tumor formation. Moreover, based on its properties, we describe the current status of DNA methylation-related diagnostic and therapeutic

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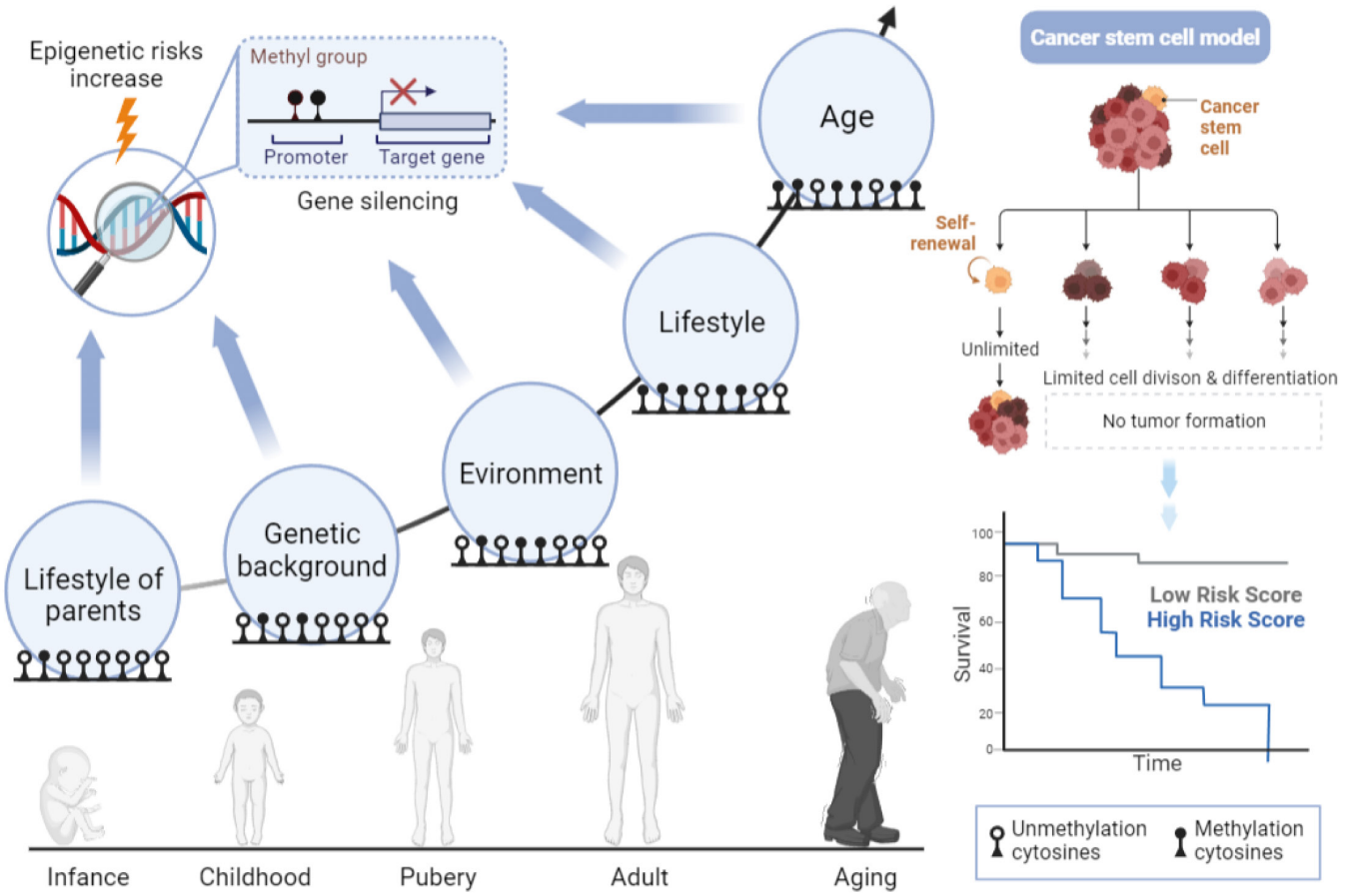



**Figure 1.** Illustration of epigenetic modifications in cancer.

applications as well as the inevitable challenges. To the best of our knowledge, we further prospect for more advanced and effective therapeutic strategies based on the combination of conventional therapeutic means and some current novel scientific technology, such as nanotechnology.

## 2. DNA methylation in cancer

Tumors tend to be classified as low, intermediate, and high grades based on their different invasive potentials, which also reflect the malignant nature of the tumor tissue. In this review, we concentrate on the division of tumors into 2 categories, benign and malignant. Noncancerous growths in the body are referred to as benign tumors. Their biological characteristics are more akin to hyperplastic tissue, which does not spread to other organs of the body. In comparison, malignant tumor cells with strong self-renewal capabilities present a more dangerous scenario. Due to their uncontrolled cell division, they continuously produce poorly differentiated offspring cells. Malignant tumors are able to disrupt the cellular connections of adjacent tissues through the enzymes they produce, enabling cellular metastasis. They may also spread or metastasize to other parts of the body via the bloodstream and lymphatic system, forming new tumor loci[24]. In the following subsections, we will summarize the research progress of DNA methylation in benign and malignant tumors, respectively.

### *2.1 DNA methylation and tumorigenesis*

In tumor cells, we have observed alterations in the modification of genetic material, some of which appear to directly influence tumor growth and other characteristics[25]. Over the past few decades, numerous studies have shown that alterations in the distribution pattern of 5-methylcytosine (5mC) can be used to distinguish cancer cells from normal cells (Figure 1). At least 3 major pathways have been identified. The first is through widespread hypomethylation of the cancer genome. The second is the local hypermethylation of tumor suppressor gene promoter regions. Third, sequences containing 5mC may undergo mutations directly through deamination, ultraviolet exposure, or other carcinogen exposures[26–28]. Notably, these 3 alterations often occur simultaneously to trigger cancer, suggesting that alterations in epigenetic homeostasis are central to cancer evolution.

Hypermethylation of promoter regions has been observed in nearly all types of human cancers. As shown in Figure 2, these hypermethylated phenomena suppress the transcription of important tumor suppressor genes, including cell cycle regulators, DNA repair proteins, and antiapoptotic factors, some of which play functional roles in normal cell development[29,30]. Substantial evidence suggests that DNA methylation observed in cancer may originate from a small subset of cells. In the early stages of human colorectal carcinogenesis, not only are target sites in normal tissues partially methylated, but target sites in polyps are also extensively modified[31,32]. Colonic epithelium is formed by adult stem cells located in crypts, and this tissue is capable of rapid self-renewal. Therefore, the methylation patterns observed in normal colon reflect those in stem cells. Although DNA methylation on individual molecules from a single crypt appears relatively uniform, methylation levels vary significantly between different crypts[33]. This suggests that within each tissue, there may be cells with very low methylation levels, while other

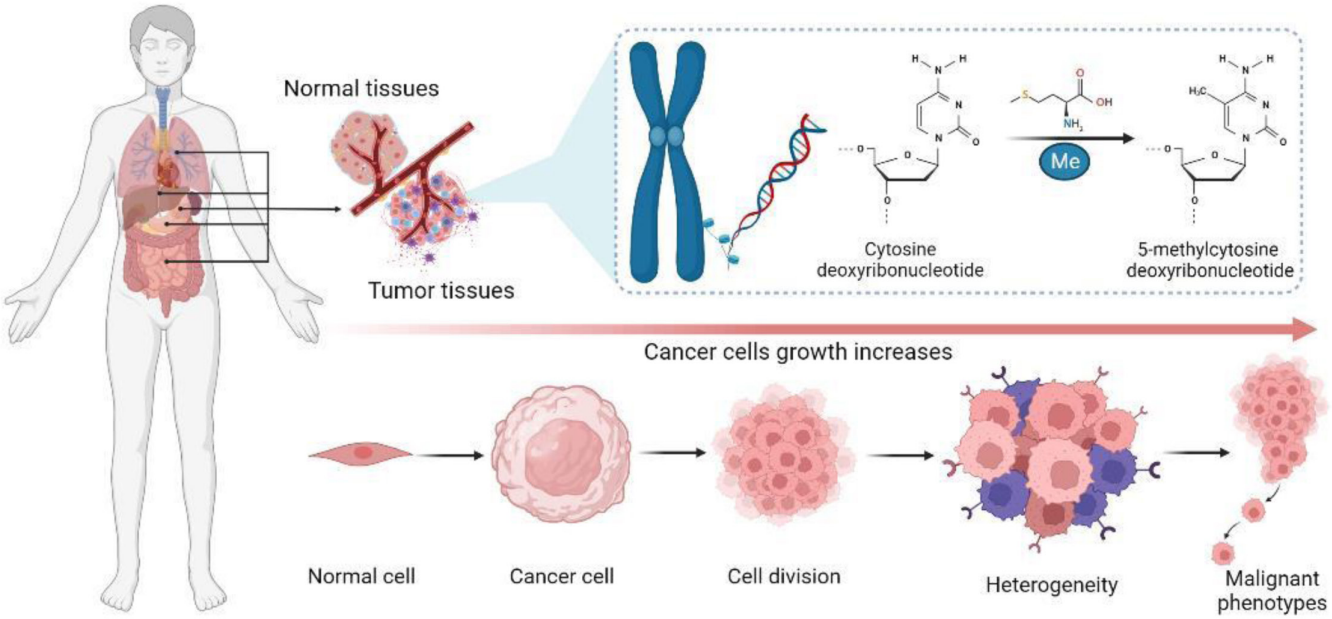



**Figure 2.** The intrinsic mechanism of DNA methylation in tumorigenesis.

cells are highly methylated in these CGI regions, promoting subsequent dominance in growth selection.

On the other hand, DNA methylation modifications also provide a potential factor for genomic mutations in tumor cells. Similar modifications may induce an irreversible constitutive heterochromatin state, but were unable to activate key differentiation genes. Therefore, these cells acquired a relatively proliferative state. While it may not be sufficient to generate tumor tissues, it is likely to provide the necessary circumstances for cells undergoing transformation through prior genetic susceptibility or spontaneous mutations. The concept that these cells accumulate DNA methylation during the aging process and become priority targets for transformation is supported by the following observations. Hematopoietic stem cells from humans indeed undergo a natural aging process, characterized by an increase in cell number and a decline in lymphoid differentiation capacity, ultimately resulting in a myeloid-dominant phenotype[34]. This aging process is associated with methylation and demethylation events commonly observed in tumors[35]. This suggests that these methylation changes may exert a role in promoting stem cell renewal and inhibiting differentiation. The most significant and earliest identified change in DNA methylation patterns in cancer cells is a reduction in methylated regions[36], which is currently classified as genome-wide hypomethylation through genome-wide analysis[37–39]. Although the full consequences of these losses remain to be clarified, DNA demethylation may lead to genomic instability and increased chromosomal aneuploidy, which are hallmark features of cancer.

Indeed, DNA hypomethylation actively contributes to increased chromosome fragility[40,41]. The loss of DNA methylation appears to be coupled with transcriptional activation, facilitating the transcription of repetitive sequences, transposable elements, and oncogenes[42,43]. Comparatively, the most well-known mechanism by which DNA methylation drives cancer is focal hypermethylation of tumor suppressor gene promoters. Typically, DNA hypermethylation occurs in CpG-rich regions or CGIs near the transcriptional start sites of abnormally silenced genes. In tumors, promoter hypermethylation can disrupt hundreds of genes, a mechanism that holds for almost all types of cancer[25,26,28,44]. In fact, through more in-depth DNA methylation analysis of various tumor types, the incidence of epigenetic changes seems to exceed the number of genetic mutations in human tumors[45,46]. Furthermore, promoter regions' hypermethylation appears in almost all genes involved in signaling pathways concerning tumorigenesis. The participation of such a massive number of genes presents an unprecedented challenge in the field of cancer epigenetics: which silencing events are truly critical to the tumorigenesis process? Detecting the critical role of each gene in tumorigenesis and progression through functional knockout experiments is a challenging task.

### 2.2 DNA methylation facilitating malignant tumor phenotypes

With the progress and development of society, the contradiction between tumor diseases and human health has become increasingly prominent. Cancer has become the principal cause of mortality in 172 countries worldwide[47]. In China, cancer has gradually developed into one of the most common causes of death among the general public and a serious public health problem. Lung cancer, colorectal cancer (CRC), gastric cancer, liver cancer, and breast cancer were the 5 most common malignancies, accounting for 57.4% of recent cancer cases, meanwhile, the 5 cancers with the highest mortality rates were, respectively, lung cancer, liver cancer, gastric cancer, CRC, and esophageal cancer, accounting for 69.3% of all deaths[48]. As a malignant disease has not been cured, epigenetic modifications, especially DNA methylation, exert a critical and nonnegligible role in many factors that promote its development. In this subsection, we summarize some recent research advances on DNA methylation associated with common malignant tumors.

#### 2.2.1 Lung cancer

Lung cancer is the most common cancer with a high mortality rate, exhibiting a significant gender preference, possibly because it is closely related to an individual's lifestyle. Previous studies have reported that CGIs of some specific genes are frequently methylated in lung cancer, including *CDKN2A*, *RASSF1A*, *RARβ*, *GSTP1*, *APC*, *DAPK*, and *TIMP3*[48–53]. The frequency of CGI methylation in specific genes in malignant tumors typically ranges from 10% to 80% (Figure 3A, B). Studies have shown that methylation of the *SHOX2* and *RASSF1A* genes is strongly associated

with lung cancer[54–56]. Kneip et al.[57] found that *SHOX2* gene methylation demonstrated 90% specificity and 60% sensitivity in distinguishing normal tissues from lung cancer. Meanwhile, Schmidt et al.[55] analyzed that *SHOX2* gene methylation appears more suitable for diagnosing lung adenocarcinoma and small cell lung cancer (SCLC), with diagnostic sensitivities of 82% and 97%, respectively. The *RASSF1A* gene is an experimental tumor suppressor gene whose methylation is closely associated with non-SCLC (NSCLC)[58,59]. Furthermore, researchers extracted lung tissue from normal subjects, NSCLC patients with cancer metastasis, and NSCLC patients without cancer metastasis for comparison (Figure 3C). Yu et al.[60] found that the *EPHB6* gene undergoes methylation in lung cancer patients, and the higher the methylation level of the *EPHB6* gene, the higher the risk of cancer metastasis. Besides, Ma et al. found that the *TMEM88* gene exhibits hypermethylation in 12 NSCLC samples. Compared with the corresponding 12 noncancer samples, patients with higher methylation levels had shorter survival times[61]. Analysis suggests that the *TMEM88* gene may act as a tumor suppressor gene, with its methylation closely associated with poor prognosis in NSCLC. These studies imply that methylation of tumor suppressor genes is an essential factor in the progression of lung cancer, indicating that demethylation of tumor suppressor genes can prevent the malignant development of lung cancer.

### 2.2.2 Colorectal cancer

Colorectal cancer is the third most common malignancy worldwide, which has a high mortality rate. Alterations in gene expression, including the activation of oncogenes and the inactivation of tumor suppressor genes, are the primary causes of CRC formation and progression[66,67]. In addition to cumulative changes in DNA sequences, the dysfunction of epigenetic regulatory systems also results in abnormal formation of carcinogenesis in colonic epithelial cells[68,69]. Recent studies have reported the differences in DNA methylation between primary and metastatic cancers (Figure 4A-D)[70,71]. The *CDKN2A* gene is more frequently methylated in poorly differentiated[72], lymph node-metastatic CRC[73], and advanced CRC[74]. Methylation of the APC and ESR1 promoters may significantly affect the metastatic potential of colon cancer. Methylated APC is more common in liver metastasis than in primary CRC[75], while ESR1 methylation has been detected in lymph nodes resected from Union for International Cancer Control stage I and II CRC patients[76]. Hibi et al. observed that HACE1 functions as an E3 ubiquitin ligase with the potential to confer a malignant phenotype. An association has been noted between increased methylation of HACE1 and tumor size. Interestingly, tumors with methylated HACE1 tend to preferentially undergo lymph node metastasis[77]. Specifically, metastatic tumor cells utilize DNA hypermethylation to suppress the expression of specific tumor suppressor genes to evade cell apoptosis and acquire a metastatic phenotype. Moreover, INF regulatory factor 8 (IRF8) is a transcription factor of the IRF family, known as a regulator of the Fas-mediated apoptosis pathway and a metastasis suppressor in solid tumors[78]. It is silenced through epigenetic mechanisms in various cancers, including CRC[79]. The IRF8 promoter is methylated in metastatic CRC cell lines but not in primary

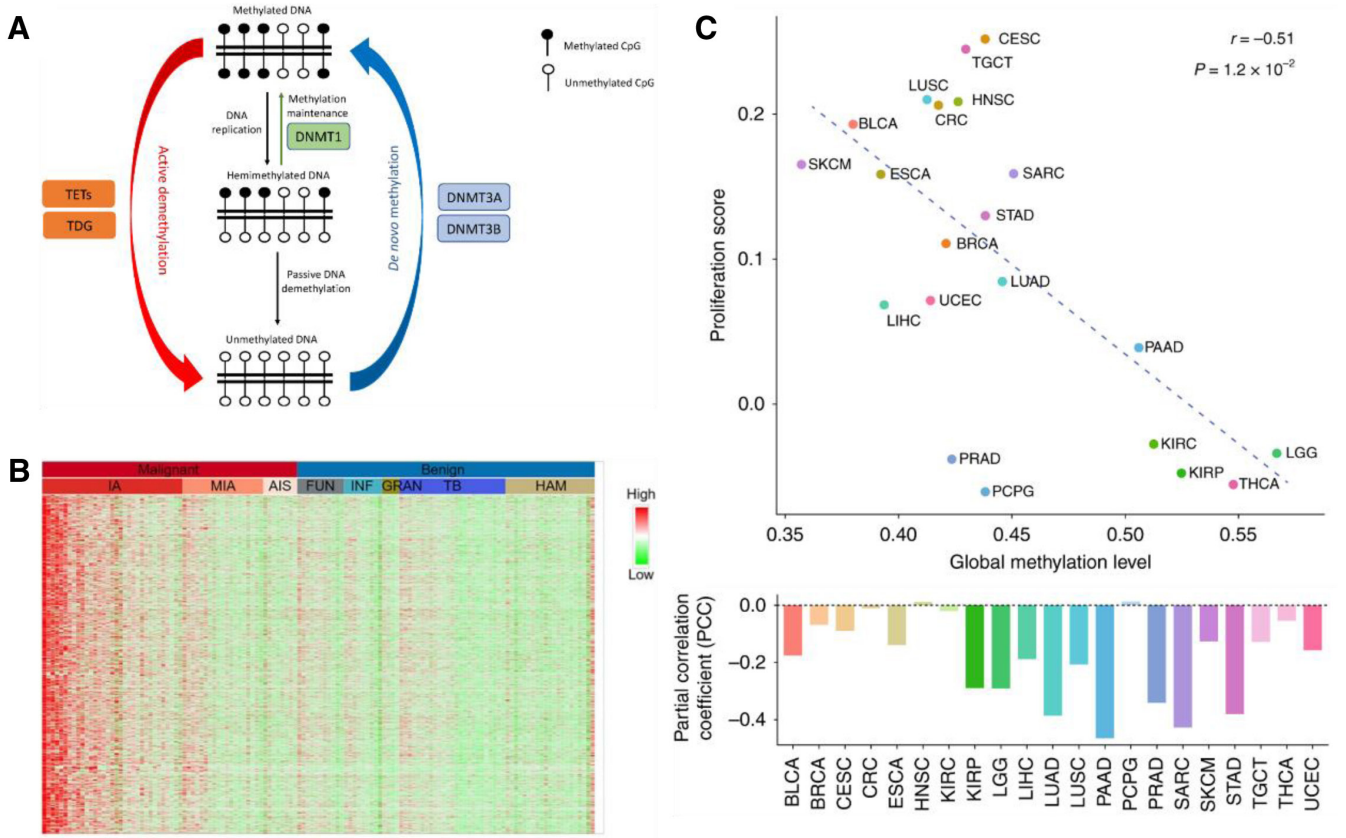



**Figure 3.** Patterns of DNA methylation in lung cancer. (A) Schematic illustration of DNA methylation dynamics of DNMTs and TETs[62]. Copyright 2022, MDPI. (B) Heatmap showing randomly selected 1000 hypermethylation regions for representative lung cancer and benign tissue samples. Subtypes from left to right are IA ($n$ = 33), MIA ($n$ = 19), AIS ($n$ = 8), FUN ($n$ = 11), INF ($n$ = 9), GRAN ($n$ = 4), TB ($n$ = 25), and HAM ($n$ = 21) [63]. Copyright 2019, Ivyspring International Publisher. (C) Correlation between genomic methylation levels and cell proliferation markers[64]. Copyright 2019, Springer Nature. TDG, AIS, adenocarcinoma in situ; FUN, fungal infection; GRAN, inflammatory granuloma; HAM, hamartoma; IA, invasive adenocarcinoma; INF, inflammation; MIA, minimally invasive adenocarcinoma; TB, tuberculosis; TDG, thymine–DNA glycosylase; TETs, 10 to 11 translocation enzymes

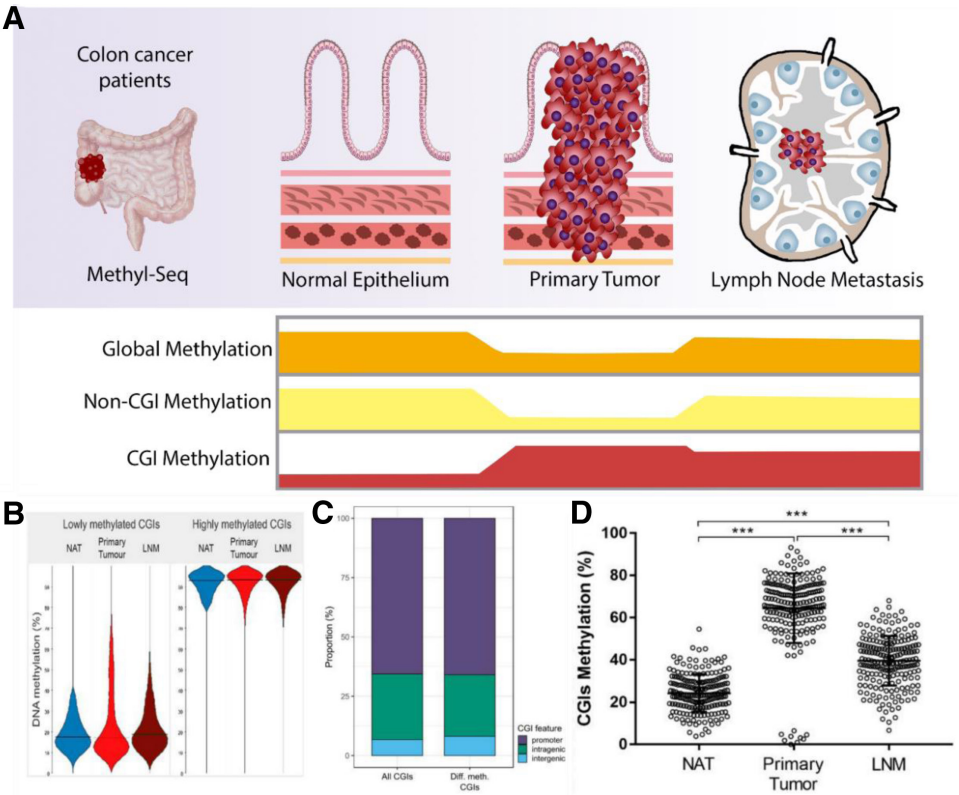



**Figure 4.** Modes of DNA methylation within CRC. (A) Methylation levels of global, non-CGIs, and CGIs at different periods in CRC. (B) CGI methylation levels in NAT, primary tumor, and LNM samples of CRC patients. (C) The proportion of different CGI features represented in all analyzed CGIs ($n$ = 7009) and differentially methylated CGIs ($n$ = 246). (D) Methylation percentage of 204 CGIs in NAT, primary tumor, and LNM samples (1.5 kb upstream to coding genes) (***$P$ < 0.001, Kruskal–Wallis followed by Dunn test)[65]. Copyright 2020, MDPI. LNM, lymph node metastasis; NAT, normal adjacent tissue.

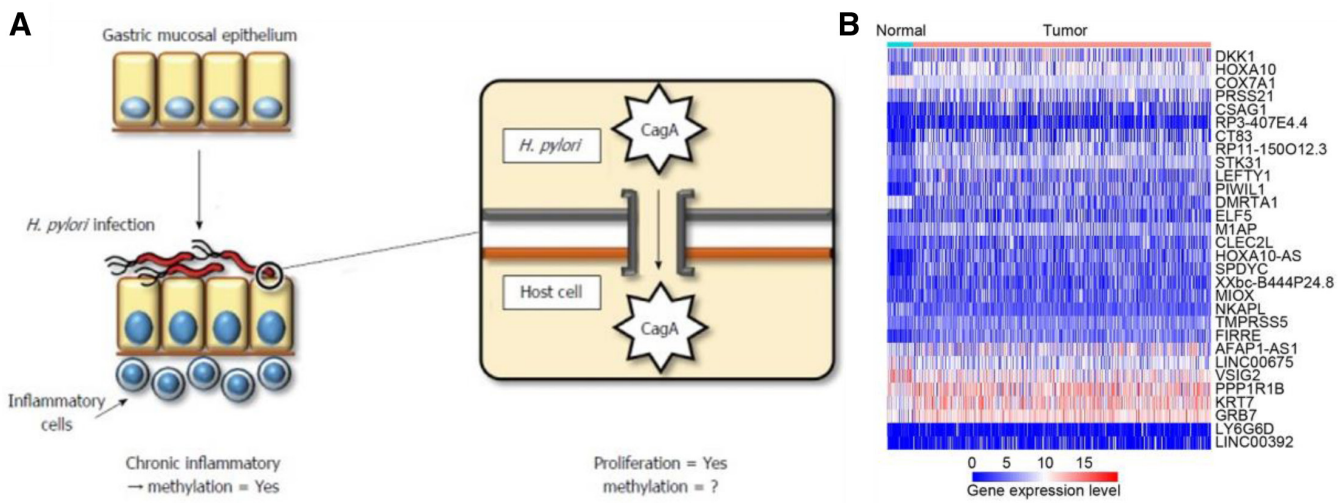
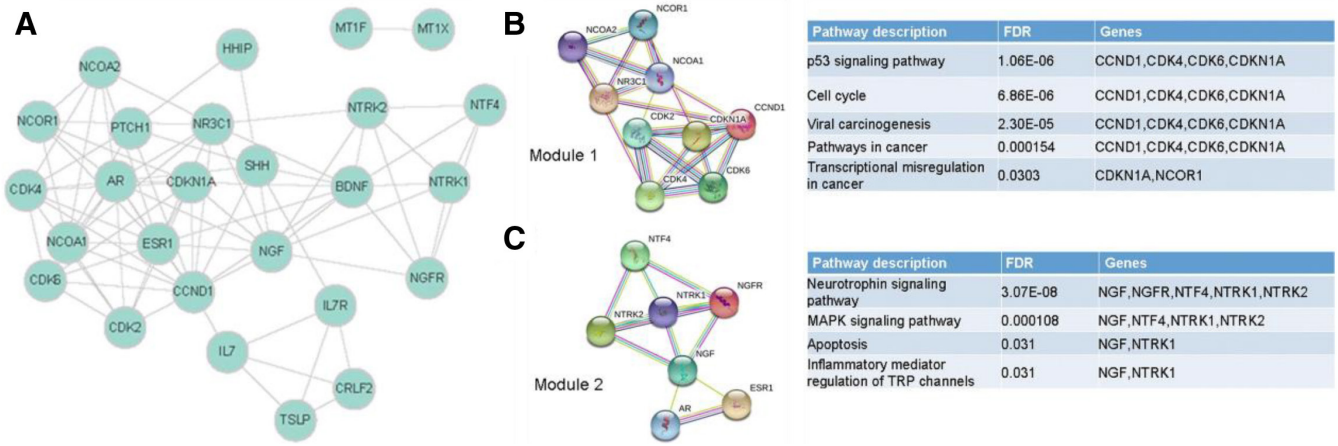



**Figure 5.** DNA methylation in gastric cancer. (A) Infectious condition and pathogenicity of Helicobacter [81]. Copyright 2014, WJG Press. (B) The expression profile of the most significant 30 methylation-related differentially expressed genes between normal ($n$ = 32) and gastric cancer samples ($n$ = 375)[82]. Copyright 2020, BioMed Central.

CRC cells. Its gene expression is negatively correlated with antiapoptotic and metastatic phenotypes in vitro[78]. These results suggest that metastatic tumors employ DNA hypermethylation to suppress IRF8 expression, thereby evading apoptotic cell death and acquiring a metastatic phenotype. *RARRES1* is also a tumor suppressor gene whose expression is frequently downregulated in various malignant tumors due to DNA hypermethylation. Downregulation of *RARRES1* is thought to be associated with the progression of CRC[80]. However, while many genes are inactivated in CRC cell lines, there is significant variability between different cell lines, suggesting that not all inactivated genes are directly related to tumorigenesis.

### 2.2.3 Gastric cancer

Gastric cancer is one of the most common malignant tumors worldwide and a leading cause of cancer-related deaths in Asia and certain European countries[83]. It has been well-known that




| Pathway description | FDR | Genes |
|---|---|---|
| p53 signaling pathway | 1.06E-06 | CCND1,CDK4,CDK6,CDKN1A |
| Cell cycle | 6.86E-06 | CCND1,CDK4,CDK6,CDKN1A |
| Viral carcinogenesis | 2.30E-05 | CCND1,CDK4,CDK6,CDKN1A |
| Pathways in cancer | 0.000154 | CCND1,CDK4,CDK6,CDKN1A |
| Transcriptional misregulation in cancer | 0.0303 | CDKN1A,NCOR1 |

| Pathway description | FDR | Genes |
|---|---|---|
| Neurotrophin signaling pathway | 3.07E-08 | NGF,NGFR,NTF4,NTRK1,NTRK2 |
| MAPK signaling pathway | 0.000108 | NGF,NTF4,NTRK1,NTRK2 |
| Apoptosis | 0.031 | NGF,NTRK1 |
| Inflammatory mediator regulation of TRP channels | 0.031 | NGF,NTRK1 |

**Figure 6.** Genes affected by methylation in HCC. (A) PPI network for hypermethylated genes. (B and C) Relative signaling pathways affected by methylation genes[96]. Copyright 2018, Cambridge University Press. HCC, hepatocellular carcinoma; PPI, Protein-Protein Interaction.

the main etiological risk factor for gastric cancer is *Helicobacter pylori* infection[84]. Previous studies have suggested that chronic inflammation induced by *H. pylori* infection may lead to abnormal DNA methylation, rather than bacteria themselves (Figure 5A)[85]. Another study utilizing the Mongolian gerbil model further confirmed that both *H. pylori* infection and abnormal methylation of the gastric mucosa can promote the development of gastric cancer[86]. Several studies have also provided evidence that epigenetic alterations facilitate gastric tumorigenesis. Various tumor suppressors and tumor-associated genes, including *APC*, *CDH1* (*E-cadherin*), *CHFR*, *DAPK*, *GSTP1*, *p16*, and *RUNX3*, are known to be silenced in gastric cancer due to hypermethylation[87–89] In addition, methylation is frequently observed in the precancerous stages of gastric cancer, suggesting that aberrant methylation occurs early in the multistep process of gastric carcinogenesis[90–92]. Accumulation of aberrant methylation is thought to promote carcinogenesis by activating common cancer pathways (Figure 5B). Some negative regulators of Wnt signaling, including SFRP1, DKK2, and WIF1, are frequently methylated in gastric cancer[[93,94]].Methylation of RASSF family genes is considered an alternative mechanism to replace KRAS mutations in signaling pathways, leading to the activated Ras signal pathway[95]. Commonly, the development of gastric cancer results from a combination of external factors, infection, and epigenetic inheritance, whose in-depth study is critical to the survival of gastric cancer patients.

### 2.2.4 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver, which is the third leading cause of cancer-related deaths for all humankind[97]. An increasing trend in the incidence and mortality rates of HCC has been observed in most countries[98]. It is well known that high methylation of the *E-cadherin* gene is commonly reported in HCC cases[99,100]. Jiang et al. demonstrated that high methylation of the CpG sites in the *E-cadherin* gene promoter region is associated with multidrug resistance in HCC. Indeed, E-cadherin expression is implicated in altered doxorubicin uptake, reduced P-glycoprotein expression, and promotion of apoptosis. Therefore, inhibition of E-cadherin facilitates cancer cells' evasion of apoptosis and acquisition of multidrug resistance (Figure 6A)[101]. Another pathway participating in HCC development is the Wnt/β-catenin signaling pathway. Hypermethylation of regions encoding Wnt regulatory factors (such as SRY box 1 [SOX1] and SRY box 17 [SOX17]) leads to aberrant activation of the Wnt/β-catenin signaling pathway, thereby enhancing the likelihood of cell proliferation and survival[100,102,103]. Shu et al. found that the *PRDM5* gene was silenced in 63% of HCC cases. As a stress response gene, *PRDM5* counteracts the Wnt/β-catenin signaling pathway, which acts as an epigenetic modifier to suppress the expression of multiple oncogenes[104]. Additionally, SFRP1 is another antagonist of the Wnt/β-catenin pathway, and its methylation levels are higher in HCC specimens than in nontumor tissues. Epigenetic suppression of SFRP1 in HCC results in enhanced cellular proliferative potential and overexpression of oncogenes such as c-Myc and cyclin D1[105]. Growing evidence supports that methylation profiling of HCC reveals significant differences between tumorous liver tissue and adjacent noncancerous liver tissue (Figure 6B, C)[100]. Multiple studies have reported aberrant hypermethylation of some tumor suppressor genes (for instance, *GSTP* and *SOCS1*) in HCC, which leads to the loss of cell cycle checkpoints and activation of cell proliferation[106,107]. During hepatocarcinogenesis, many tumor suppressor genes are silenced by DNA methylation, for which hypermethylation of CGIs at promoters is an important mechanism for their inactivation.

### 2.2.5 Breast cancer

The progression of breast cancer is the result of a multistage carcinogenic process. Clinical data indicate that breast cancer begins as a less invasive hormone-dependent type, which gradually progresses to a highly aggressive hormone-dependent phenotype (Figure 7A-C)[111]. Among the methylated genes in breast cancer, there are plenty of tumor suppressor genes, such as *p16*, whose methylation is reported to silence the gene and block cell growth regulatory signals[112,113]. Methylation of *p16* prepared from the plasma of breast cancer patients has been reported to be involved in the lymph node metastasis process[114]. Damage response genes are another type of methylated genes reported in breast cancer, such as *BRCA1*[115] and mismatch repair genes *hMLH1* and *hMSH2*[116]. Disruption of repair genes may elevate sporadic mutation frequency, a hallmark of tumorigenesis. Moreover, members of the steroid receptor gene family, including the estrogen receptor[117] and *RARβ2* receptors, have been reported to undergo methylation in a subset of breast cancer cases[118]. The interaction of *RARβ2* receptor with retinoic acid may resist tumor proliferation, whose silencing provides a selective benefit to late-stage breast cancer cells. *E-cadherin* and *TIMP3* have also been detected to be methylated in breast cancer, thereby facilitating the metastasis of breast cancer cells[119,120]. *APC* gene, as a tumor suppressor gene with multiple cellular functions, plays a crucial

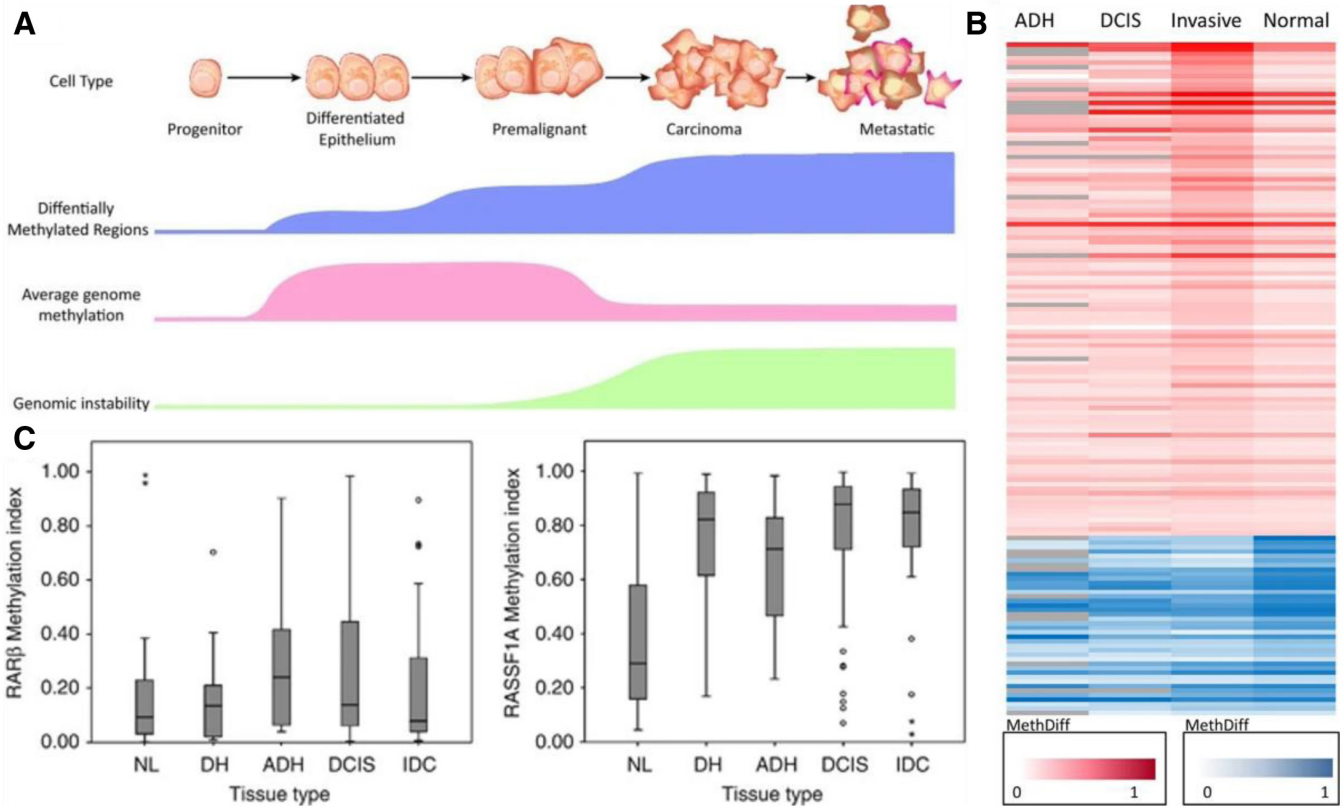



**Figure 7.** DNA methylation in breast cancer. (A) Progression of breast cell epigenome from progenitor cells to malignancy[108]. Copyright 2012, BioMed Central. (B) Differentially methylated genes in ADH, DCIS, and invasive breast cancer compared with healthy tissue[109]. Copyright 2020, Springer Nature. (C) *RARβ2* and RASSF1A vs tissue type (normal breast epithelia [NL], ductal hyperplasia [DH], atypical ductal hyperplasia [ADH], ductal carcinoma in situ [DCIS], and invasive ductal cancer [IDC])[110]. Copyright 2013, Springer Nature.

role in the Wnt signaling pathway, cell–cell adhesion, and apoptosis[121]. *DAPK1* is a proapoptotic serine/threonine protein kinase gene, acting as a positive mediator of γ-interferon (IFN-γ) mediated programmed cell death[122]. *GSTP1* is a gene that plays a key role in detoxifying exogenous substances, carcinogens, pesticides, and various environmental pollutants. In the MAPK signaling pathway, *GSTP1* participates in the regulation of cell survival and death signals[123]. Phosphatase and tensin homolog (PTEN), acting as a dual lipid and protein phosphatase, regulates the PI3K/AKT signaling pathway through its target PIP3 in breast cancer[124]. *DCR1* and *DCR2* are genes encoding membrane receptors that bind TNF-related apoptosis-inducing ligand and inhibit the TNF-related apoptosis-inducing ligand apoptosis pathway[125]. Through extensive analysis of clinical samples, researchers discovered that the aforementioned tumor-related genes were abnormally methylated in breast cancer cells. For the reason that tumorigenesis involves the coordinated expression and repression of multiple genes, there must be common mechanisms responsible for coordinating these changes. Understanding the mechanisms involved in this project is a considerable issue in breast cancer biology and treatment.

### *2.2.6 Others*

Following the rapid advancement of epigenetics research in recent years, several unique DNA methylation patterns have been identified as playing a significant role in the progression of malignant tumors. The first type involves alterations in DNA methylation patterns caused by gene mutations. In 2010, Noushmehr et al. defined a CGI methylator phenotype (G-CIMP) in glioblastoma. The methylation pattern is highly correlated with mutations in isocitrate dehydrogenase 1 (IDH1) in tumor cells[126]. Mutated IDH1 catalyzes the conversion of α-ketoglutarate (α-KG) to D-2-hydroxyglutarate, structurally similar compounds. The latter competitively inhibits α-KG-dependent epigenetic modifiers (eg, histone and DNA demethylases), leading to genome-wide hypermethylation[127]. Studies indicate that G-CIMP$^+$ patients tend to be younger, exhibit longer overall survival, and possess favorable prognostic expectations[128]. IDH1 mutations are also prevalent in hematologic malignancies, including myelodysplastic syndrome (MDS) and T-cell lymphoma[129,130], as well as solid tumors such as chondrosarcoma[131] and cholangiocarcinoma[132]. Meanwhile, dysregulation of non-coding RNAs (ncRNAs) has also been identified as a vital factor influencing multiple cellular characteristics in tumor cells. The ncRNAs are able to exert a synergistic effect by interregulating with DNMT3B to influence the progression of tumor cells[133]. Recent comprehensive studies indicate that the miR-29 family plays a pivotal role in the pathogenesis of glioma[134]. Xu et al. demonstrated that DNMT3B expression in the U87MG glioblastoma cell line was effectively inhibited by miR-29-mediated mRNA degradation. Exogenous miR-29 introduction significantly impedes U87MG cell proliferation and migration while stimulating apoptosis[135]. Wang et al.[136] reached similar conclusions when investigating the relationship between miR-29 and DNMT3B in pancreatic cancer. Besides that, the event that DNA methylation induced by infected viruses promotes tumor cell progression is gradually confirmed. Viral infection also induces aberrant DNA methylation in the human genome leading to carcinogenesis[137], such as HCC related to hepatitis B virus and hepatitis C virus[138] as well as cervical carcinoma[139] and head and neck squamous cell carcinoma[140], both of which are associated with human papillomavirus. Most of these aberrant

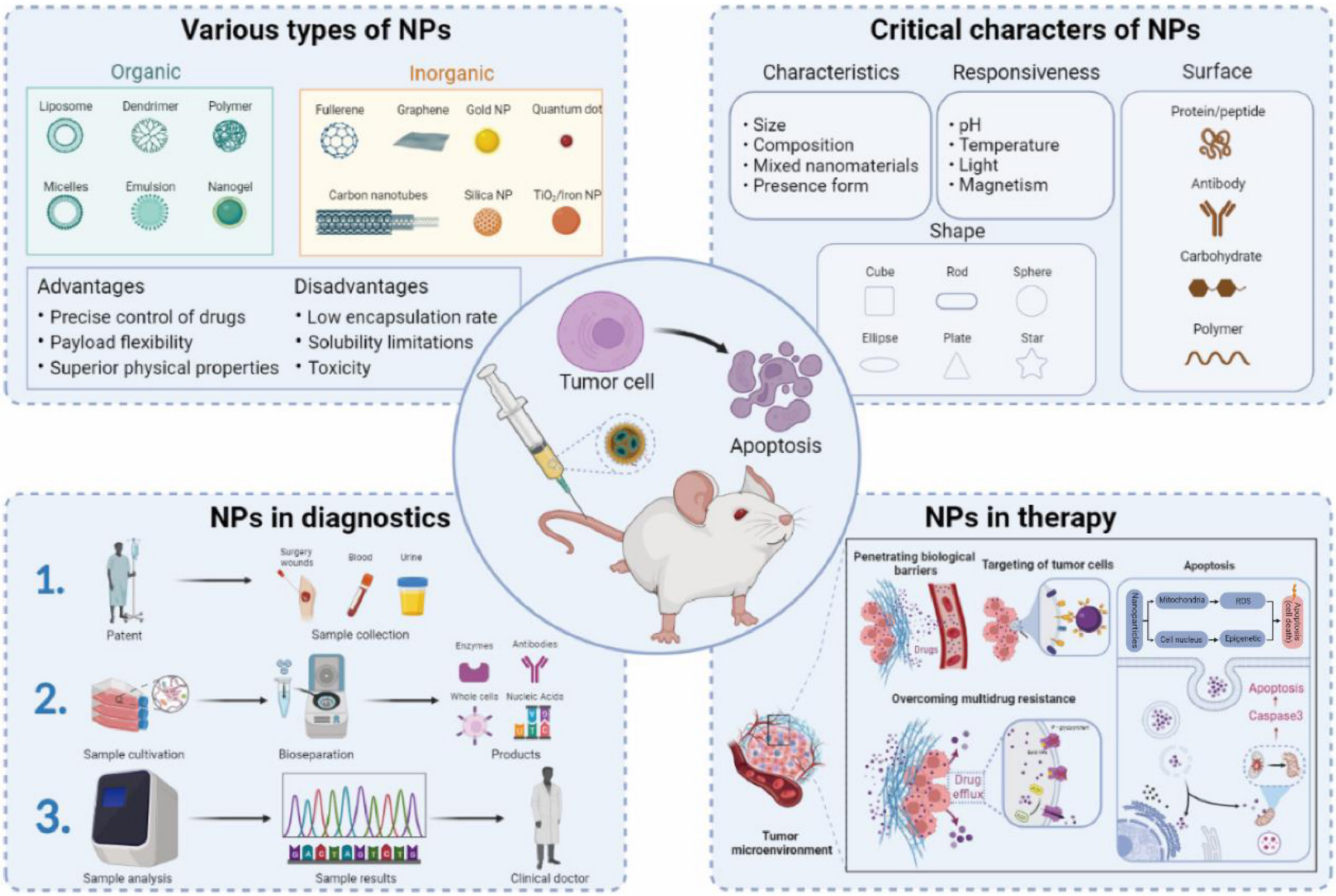



**Figure 8.** Overview of nanomedicine in clinical practice for cancer diagnosis and treatment.

DNA methylation events are induced by viral infection. Consequently, understanding the mechanisms of epigenetic regulation in cancer therapy is critical for optimizing existing therapeutic strategies, improving the prognosis of cancer patients, and exploiting the reversible properties of epigenetic modifications against cancer (Table 1).

## 3. Present therapeutic strategies for tumors associated with DNA methylation

### *3.1 DNA methylation-related diagnosis*

The occurrence and development of cancer are accompanied by various epigenetic alterations, including gene methylation and mutation[236–238]. The discovery that tumors can release epigenetic products into the bloodstream garnered significant attention, making it possible to utilize them as noninvasive liquid biopsy biomarkers for early cancer diagnosis[239]. As aberrant DNA methylation usually occurs early in cancer, DNA methylation has been recognized as the most promising biomarker for cancer diagnosis and prognostic monitoring[240,241]. Some of the important methods for detecting DNA methylation, such as bisulfite-converted DNA-based techniques, methylation-sensitive restriction endonuclease methods, CRISPR-based biosensor methods, and third-generation sequencing methods, are discussed in this subsection.

#### *3.1.1 Bisulfite sequencing*

Bisulfite sequencing is regarded as the gold standard for detecting DNA methylation, widely used for this purpose[243]. The method is based on the fact that bisulfite specifically converts unmethylated cytosine to uracil, while methylated cytosine is unaffected. After bisulfite treatment, analysis of cytosine residues in DNA sequences by polymerase chain reaction (PCR) amplification and sequencing is able to distinguish between methylated and unmethylated cytosines[244,245]. The key advantage of this method is its high sensitivity and resolution, which enable the detection of low-frequency methylation events. Thus, it is widely used for genome-wide methylation analysis, especially for the study of gene regulation, disease epigenetics, and changes in gene methylation during development. Although this technique has achieved remarkable results in many fields, its drawbacks should not be ignored, including the complexity and high cost of the experimental process, and the need to strictly control the experimental conditions to avoid false-positive or false-negative results.

#### *3.1.2 Methylation-sensitive restriction enzymes–based methods*

The method based on methylation-sensitive restriction enzymes (MSREs) is one of the classical techniques for detecting DNA methylation. The method relies on the cleavage properties of certain restriction endonucleases, which are sensitive to methylated cytosine and are able to recognize and cleave unmethylated DNA sequences, but not methylated DNA[246,247]. This property makes the MSRE method an important tool for analyzing DNA methylation. Usually, the operation process of the MSRE assay includes DNA sample extraction, enzymatic reaction, PCR amplification, and subsequent analysis. By this method, the methylation level of a specific gene region or the

**Table 1**

**Genes with aberrant methylation in various cancers.**

| Gene | Tumor type | Molecular regulation | Function | References |
|---|---|---|---|---|
| CDKN2A | Lung cancer, breast cancer, colorectal cancer, gastric cancer, thyroid cancer, hepatocellular carcinoma, bladder cancer, cervical cancer, leukemia | Hypermethylation | Cell cycle regulation, tumor metastasis | [48,88,141–147] |
| E-cadherin | Breast cancer, gastric cancer, hepatocellular carcinoma, cervical cancer, bladder cancer | Hypermethylation | Tumor metastasis, drug resistance | [88,100,119,142,148] |
| RAR$\beta$ | Lung cancer, oropharyngeal squamous cell carcinoma, cervical cancer, colon cancer | Hypermethylation | Tumor suppressor genes | [12,149–151] |
| MGMT | Glioblastoma multiforme, colorectal cancer, pancreatic cancer | Hypermethylation | Drug resistance | [152–155] |
| SEPT9 | Gastric cancer, colorectal cancer, prostate cancer, cervical cancer, liver cancer | Hypermethylation | Diagnostic marker | [156–160] |
| SHOX2 | Prostate cancer, lung cancer, melanocytoma | Hypermethylation | Diagnostic marker | [57,158,161,162] |
| VIM | Urothelial carcinoma, bladder cancer | Hypermethylation | Diagnostic marker | [163,164] |
| DAPK | Lung cancer, breast cancer, gastric cancer, thyroid cancer, cervical cancer | Hypermethylation | Tumor suppressor genes | [50,88,165–167] |
| RASSF1A | Lung cancer, breast cancer, thyroid cancer, gastric cancer, bladder cancer | Hypermethylation | Tumor suppressor genes | [95,142,168–170] |
| GSTP1 | Lung cancer, breast cancer, gastric cancer, hepatocellular carcinoma, prostate cancer | Hypermethylation | Cell survival | [50,88,171–173] |
| APC | Lung cancer, colorectal cancer, breast cancer, cervical cancer | Hypermethylation | Tumor suppressor genes | [52,75,174,175] |
| PTEN | Breast cancer, thyroid cancer, hepatocellular carcinoma | Hypermethylation | Tumor suppressor genes | [166,171,176] |
| PAX1 | Cervical cancer, oral squamous cell carcinoma, esophageal squamous cell carcinoma, parathyroid adenomas | Hypermethylation or hypomethylation | Tumorigenesis | [177–181] |
| TIMP3 | Thyroid cancer, oral cancer, lung cancer, cavity cancer, head and neck cancer | Hypermethylation | Tumor metastasis | [182–186] |
| RUNX3 | Gallbladder cancer, renal cancer, breast cancer, prostate cancer, lung cancer | Hypermethylation | Tumor suppressor genes | [187–192] |
| SFRP1 | Colorectal cancer, ovarian cancer, pancreatic cancer, breast cancer, prostate cancer | Hypermethylation | Tumor metastasis | [193–197] |
| CDKN2B | Colorectal cancer, leukemia | Hypermethylation | Tumor suppressor genes | [147,198,199] |
| LOX | Melanoma, nasopharyngeal carcinoma | Hypermethylation | Tumor suppressor genes | [200,201] |
| SOCS1 | Prostate cancer, colorectal cancer, pancreatic cancer, liver cancer, myeloma, leukemia | Hypermethylation | Tumor metastasis | [106,202–206] |
| ESR1 | Breast cancer, ovarian cancer, bladder cancer, colorectal cancer | Hypermethylation | Diagnostic marker | [207–210] |
| TMEFF2 | Gliomas, colorectal cancer, lung cancer, bladder cancer, oral squamous cell carcinoma, breast cancer | Hypermethylation | Diagnostic marker | [193,211–215] |
| MAL | Cervical cancer, bladder cancer, cervical cancer, ovarian cancer, lymphoma | Hypermethylation | Tumor suppressor genes, drug resistance | [216–220] |
| BNC1 | Pancreatic cancer, liver cancer | Hypermethylation | Diagnostic marker | [221,222] |
| TBX2 | Bladder cancer, lung cancer | Hypermethylation | Tumor suppressor genes, drug resistance | [223,224] |
| SLIT2 | Gliomas, gastric cancer, lung cancer, breast cancer, colorectal cancer | Hypermethylation | Tumor suppressor genes | [225–230] |
| FOXL2 | Ovarian tumors | Hypermethylation | Diagnostic marker | [231] |
| PTGER4 | Lung cancer, colorectal cancer | Hypermethylation | Diagnostic marker | [232,233] |
| ALDH | Lung cancer, ovarian cancer | Hypermethylation | Diagnostic marker | [234,235] |

whole genome can be assessed quantitatively or qualitatively. Compared with other methylation detection methods, the MSRE method has a simpler operating procedure and lower cost and is therefore widely used in small-scale experiments[248]. In addition, MSRE technology is often applied in combination with other methods, such as bisulfite sequencing, DNA microarrays, or high-throughput sequencing, to improve the coverage and resolution of detection[249,250]. However, a limitation of the MSRE method is its dependence on specific restriction endonucleases, which means that it can only detect methylation sites recognized by these enzymes and cannot fully reflect the methylation status of the entire genome.

### *3.1.3 CRISPR/Cas system-based methods*

CRISPR-based biosensors for the detection of DNA methylation are a rapidly developing technology in recent years, taking full advantage of the properties of the CRISPR/Cas system in pinpointing DNA sequences[251]. Such methods mainly rely on variants such as CRISPR/Cas9, CRISPR/Cas12, or CRISPR/Cas13, which are systems capable of efficiently recognizing and cleaving specific DNA or RNA sequences. When combined with specific methylation markers, these systems can provide sensitive, specific, and efficient methylation detection[252]. Specifically, CRISPR/Cas systems recognize DNA sequences containing methylation marks by combining Cas proteins with a guide RNA that targets the methylation site. This process can be detected not only by signal outputs such as fluorescence and luminescence but also by the ability to further enhance the signal by combining it with other molecular probes, further improving the sensitivity and specificity of the detection. Currently, there are 2 typical application strategies for CRISPR-based biosensors: one is to take advantage of the cleavage activity of Cas12 or Cas13 proteins, which recognize a target sequence and cleave the single-stranded DNA or RNA next to it, thus generating an easily detectable optical signal[253]. Another approach is through the gene-editing function of the CRISPR/Cas9 system, which can trigger a downstream gene-editing reaction when a methylated DNA sequence is recognized, and then the methylation status can be sensed by fluorescence and chemical reaction. These 2 strategies are superior in terms of sensitivity, specificity, and ability to detect multiple methylation sites, making them widely applicable in epigenetic research, early disease diagnosis, and personalized medicine[254]. In addition, CRISPR-based biosensors offer greater operational simplicity and lower cost compared with traditional bisulfite sequencing methods and are

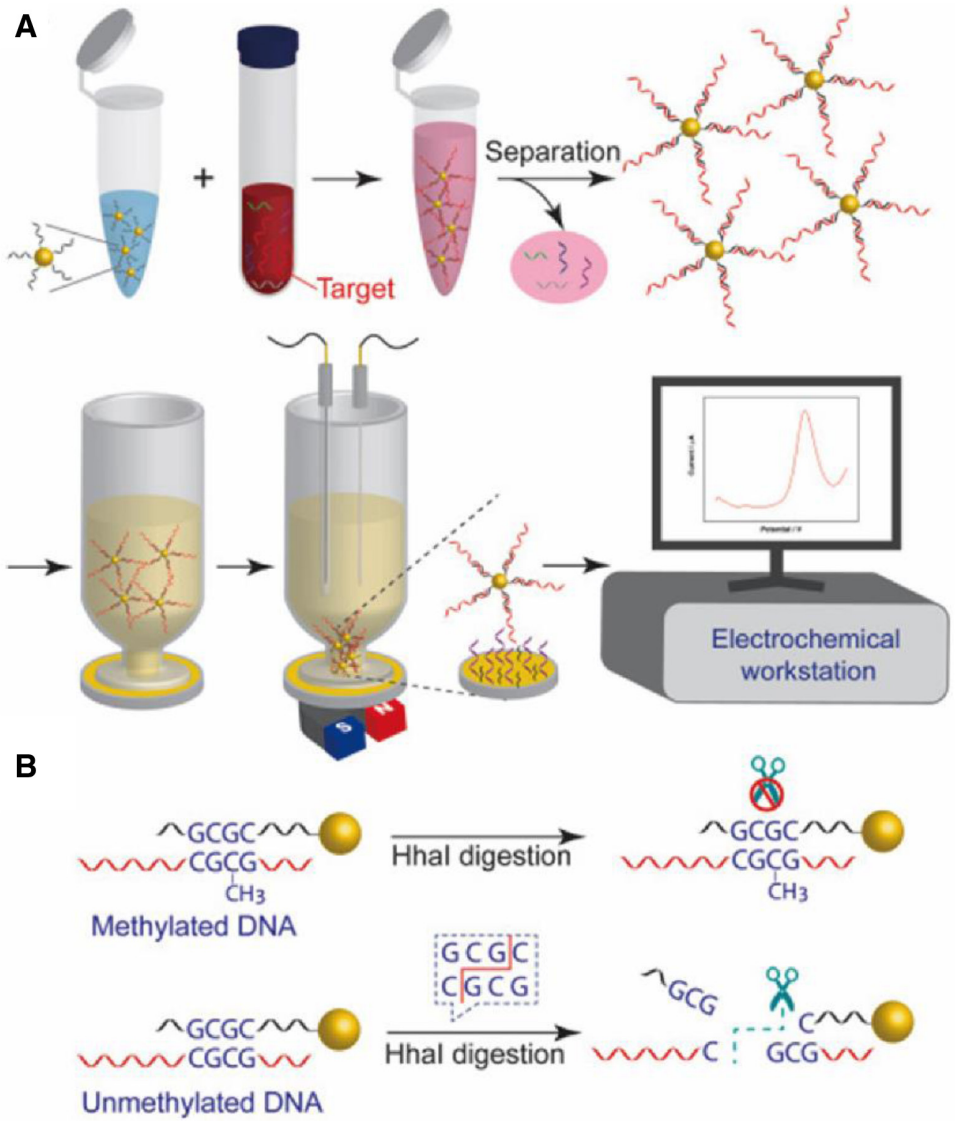



**Figure 9.** Schematic diagram of DNA methylation detection based on nucleic acid hybridization on a gold-plated magnetic nanoparticle network. (A) Methylated DNA measurement workflow. (B) Principle of DNA-specific detection of methylation[242]. Copyright 2020, Elsevier.

particularly suitable for high-throughput and rapid on-site detection. However, this technology still faces many challenges, including the stability of the system, the amplification efficiency of the signal, and how to perform efficient signal discrimination in complex samples.

### *3.1.4 Third-generation sequencing*

Third-generation sequencing–based methods have shown great potential for DNA methylation detection. Compared with traditional second-generation sequencing, third-generation sequencing has the advantages of longer read lengths, higher throughput, and real-time detection, especially in genome-wide DNA methylation analysis, which can provide a more refined and comprehensive perspective. Representative methods of third-generation sequencing technologies include single-molecule real-time (SMRT) sequencing and nanopore sequencing, both of which have unique application advantages in the detection of DNA methylation[255,256]. In SMRT sequencing, the technology developed by the PacBio Company identifies DNA sequences by sequencing in real-time at the single-molecule level and utilizing changes in the fluorescence signal of DNA polymerase. Because the SMRT sequencing technology is able to simultaneously detect differences between methylated and nonmethylated cytosines on the DNA strand, it is able to infer the methylation status directly from the signal changes without the need for tedious chemical modification steps. This allows SMRT sequencing to have extremely high resolution in methylation detection, effectively identifying rare methylation events and providing precise data to support epigenetic studies[257]. Another major third-generation sequencing technology is nanopore sequencing, with a representative platform being Oxford Nanopore Technologies[258]. Nanopore sequencing directly reads DNA sequences by measuring the changes in electrical currents induced by the passage of DNA molecules through nanopores. In the process, nanopore sequencing technology is able to directly sense how methylation modifications on the DNA strand affect the current, thus identifying the methylation status at a specific location. Compared with SMRT sequencing, nanopore sequencing technology offers greater portability and real-time performance, making it particularly suitable for clinical field testing and rapid diagnosis. By using appropriate algorithms, nanopore sequencing enables in-depth analysis of methylation patterns, further advancing its application in epigenetics and disease monitoring. Methylation detection methods based on third-generation sequencing have not only improved the

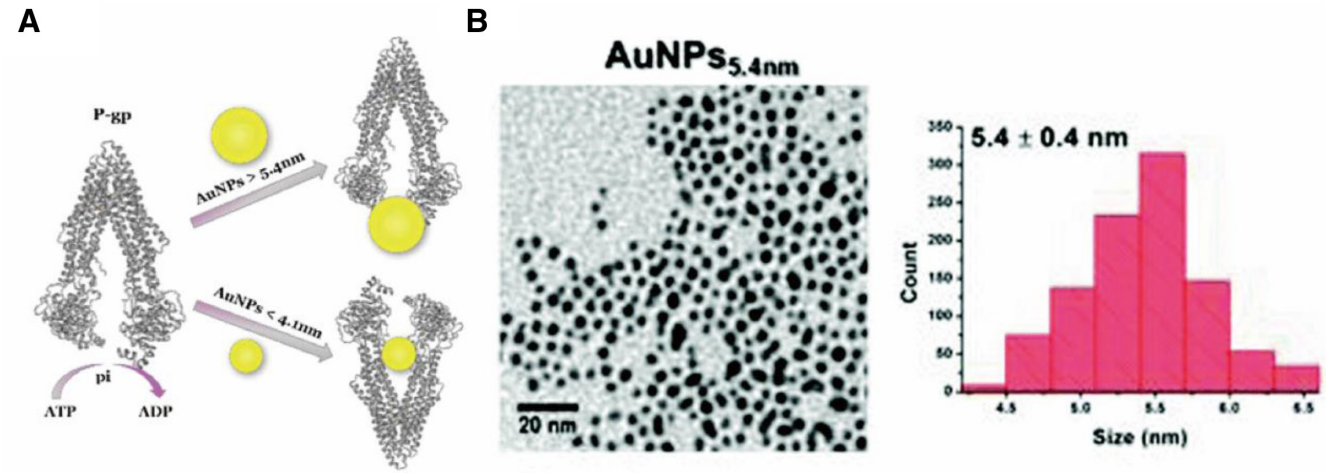



**Figure 10.** Nanomaterials for multidrug resistance. (A) Principle of gold nanoparticles against multidrug resistance. (B) Transmission electron microscopy images (TSM) and particle size distributions of 5.4 nm AuNPs. [266]. Copyright 2020, Royal Society of Chemistry. AuNPs, Au naoparticles.

accuracy of the assays but also extended their application to large-scale, complex samples. Especially in the field of oncology research and personalized medicine, third-generation sequencing technology can reveal early markers of disease and potential therapeutic targets by accurately depicting changes in methylation patterns. However, the challenges of high cost, data processing complexity, and relatively high error rate of third-generation sequencing technology remain bottlenecks in the current development of the technology. As the technology continues to mature, third-generation sequencing will play a greater role in a wider range of application scenarios in the future.

To date, most of the mainstream methods for DNA methylation analysis require PCR and sequencing[259,260]. Unfortunately, these mainstream DNA methylation analysis methods face a number of problems, including their cumbersome cloning and sequencing workflows, complex operational steps, reliance on sophisticated instruments, and high costs, which make them unable to meet the practical needs of real-time diagnosis and clinical practice. With the updating of science concepts, nanotechnology has opened up new avenues for traditional tumor diagnosis and treatment, especially for the application of epigenetic diagnosis in tumors (Figure 8)[261], such as the third-generation sequencing technology. Several physically and chemically based strategies for DNA methylation detection have been reported recently, such as DNA methylation assays based on single-base extension reactions and surface-enhanced Raman spectroscopy[262], surface plasmon resonance[263], methylation-specific microarrays[264], and methylation-specific fluorescence resonance energy transfer (Figure 9A, B)[265]. Generally, there are still some limitations in the application of nanotechnology for the identification of methylation levels in cancer patients, such as the design of detection materials, the requirements for the operation of detection equipment, and the fatal problem: the contradiction between the low detection limit and the quick analysis time. Commonly speaking, the lower the detection limit, the longer the analysis time required. Therefore, an essential milestone in applying DNA methylation detection to clinical practice is the development of rapid, highly sensitive, and selective sensing technologies for clinical samples, providing clinicians with a timely diagnostic basis to strive for more living space for patients.

### *3.2 DNA methylation-related therapy*

Presently, the main DNA methylation drugs for the treatment of tumors are demethylating agents, including 5-azadeoxyguanine (5-Aza-dC) and 5-azadeoxycytosine (5-Aza-C), which inhibit the activity of DNMTs by competitive binding to DNA sequences, reversing the abnormal DNA methylation in cancer cells. These drugs effectively decrease methylation levels in the promoter regions of tumor suppressor genes, thereby restoring the expression of these genes and inducing apoptosis in tumor cells. The demethylating agents 5-Aza-dC and 5-Aza-C have been approved for the treatment of hematologic tumors, such as acute myeloid leukemia and MDS[267]. Clinical studies have shown that these demethylating agents can significantly improve patient survival, although their side effects, such as myelosuppression and immunosuppression, remain a challenge during treatment. In addition to demethylating agents, drugs such as Vidaza (azacitidine) and decitabine have shown their potential in the clinic. By inhibiting DNMTs, these drugs reduce aberrant methylation patterns in tumor cells and activate the expression of tumor suppressor genes[268]. Tumor therapeutic drugs targeting DNA methylation have been applied mainly in hematological tumors, with relatively few applications in solid tumors[269,270]. One of the major challenges in applying these DNA methylation drugs is the specificity and durability of the treatment. Since DNA methylation alterations occur in many different genes and may result in effects on healthy cells, achieving more precise drug targeting and reducing side effects remains a focus of current research.

Nanomedicine is a research field that integrates advanced nanotechnology into medicine, with the aim of advancing disease prevention, diagnosis, and treatment[271]. Combining therapeutic agents with nanomaterials has been considered a promising future direction, as it can enhance the targeting precision of drugs at the cellular level, thereby effectively avoiding systemic side effects[272,273]. Depending on their chemical composition, these nanoparticles are able to encapsulate and protect hydrophilic or hydrophobic drugs from enzymatic, chemical, or physical degradation, maintaining stability during transport to target cells[274]. Researchers have utilized various drug-loaded nanospheres to enhance cancer treatment efficacy, including organic, inorganic, and hybrid nanoparticles (Figure 10A, B)[275–277]. The first-generation "demethylating agents" azacitidine and decitabine are the most successful epigenetic modulators, widely utilized in the clinical treatment of myeloid malignancies and highly favored as pretreatment drugs for advanced solid tumors. Unfortunately, the clinical response rate of these drugs is only 30% to 50%, and their clinical efficacy is limited by low bioavailability. The genomic instability caused by the activation of multiple oncogenes at high doses of similar drugs has led to their rare use in

the clinic, especially in solid tumors. To improve the efficacy of these drugs, the introduction of nanodelivery carriers provides better targeting and biocompatibility.

Nanodelivery systems, especially nanoparticles (eg, lipid nanoparticles and polymer nanoparticles), can effectively carry DNA methylation drugs and enhance their stability. Nanocarriers can enhance drug accumulation in tumor tissues by altering drug release kinetics and reducing drug degradation in the blood circulation (Figure 11A). Nanodelivery carriers release drugs through external or internal stimuli (eg, pH changes and temperature changes) to achieve precise drug release and delivery (Figure 11B, C)[278]. The key advantage of nanodelivery carriers over conventional drug delivery systems is that the specific recognition of tumor cells and drug release can be achieved through surface modification, thus minimizing the toxic effects on normal tissues. For example, lipid nanoparticles not only efficiently carry DNA-methylated drugs but also promote drug endocytosis through the fusion of cell membranes, thus enabling precise delivery to tumor cells[279]. In addition, polymeric nanoparticles such as poly-lactide-co-glycolide (PLGA) nanoparticles have demonstrated superior drug delivery capabilities. PLGA nanoparticles can avoid premature drug clearance by modulating their surface properties and can control the rate of drug release[280,281]. Meanwhile, PLGA nanoparticles have better biodegradability, which enables the drug to be released gradually after delivery to the target site, reducing toxic side effects[282,283]. The advantage of the nanodelivery system is not only to improve the targeting of drugs but also to solve the solubility and bioavailability of DNA methylation drugs in the body. By combining nanotechnology, the efficacy of DNA methylation drugs has been significantly improved, and the side effects of the drugs have been effectively controlled.

### 3.3 Concerns related to DNA methylation therapy

As is well known, the methylation level of the vast majority of CpG sites in the genome (excluding CpG-enriched regions) is approximately 80%. In cancer, the average CpG methylation level ranges from 40% to 60%. Advances in gene sequencing technology have enabled researchers to map the patterns of genetic modifications more precisely, which has revealed that DNA hypomethylation covers about one-third of the genome[287,288]. The exact mechanism by which DNA methylation is lost from the cancer epigenome remains poorly understood. Moreover, the widespread occurrence of DNA hypermethylation in promoter regions poses unprecedented challenges in understanding the scope of these changes. Hypermethylation of specific gene promoters alone does not necessarily imply functional significance in cancer, similar to the case of gene mutations. Particularly, the hypermethylated genes are not known tumor suppressor genes, and there is no evidence that the gene is frequently methylated in cancer. Thus, the discussed genes must be investigated to determine the significance of their functional loss, including both biological processes regulated by encoded proteins and their impact on tumor progression.

Currently, the most critical issue faced by epigenetic therapy is specificity, meaning that its effects manifest at the genome-wide level rather than at the target-specific level. It may lead to the simultaneous targeting of tumor suppressor genes while also promoting the excessive activation of oncogenes,

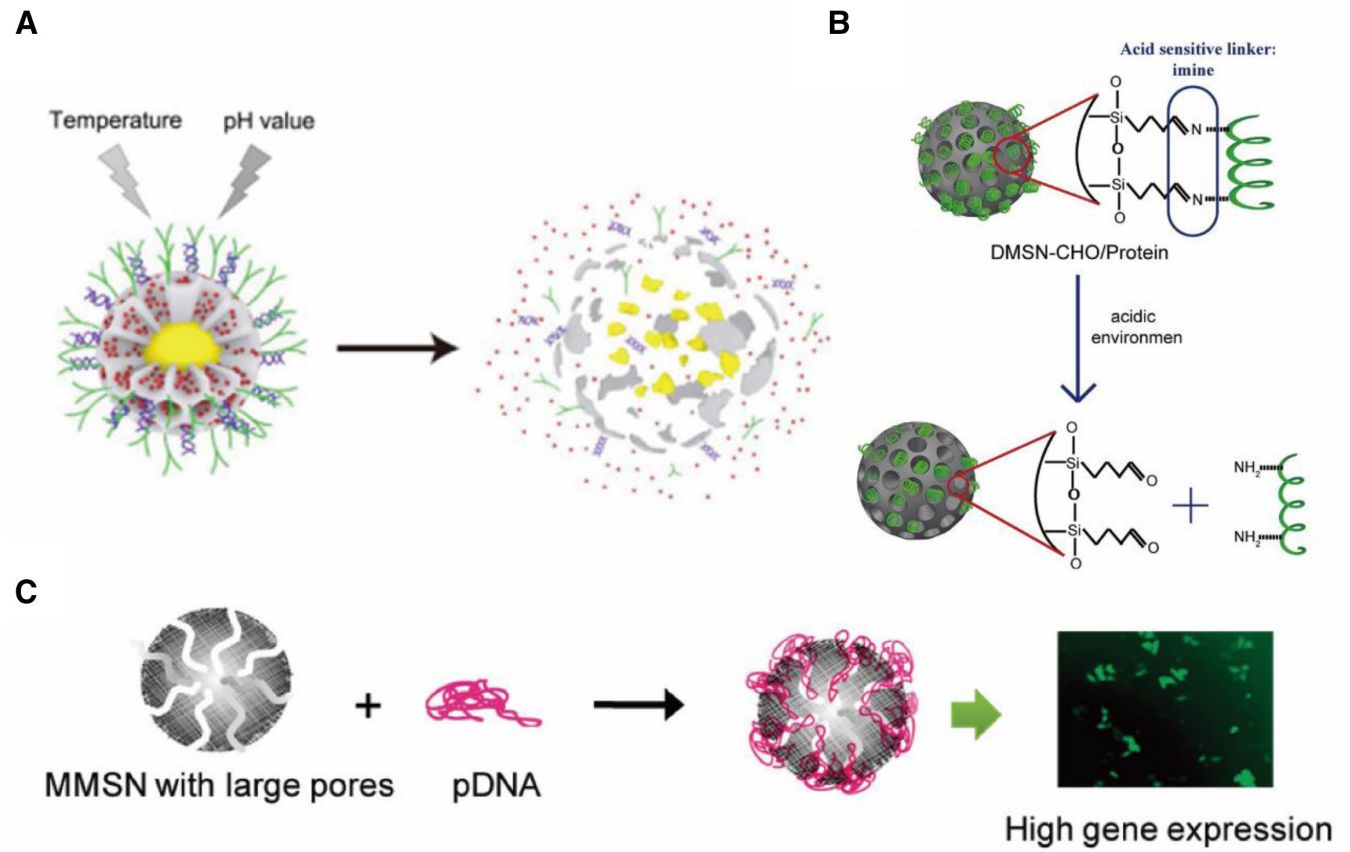



**Figure 11.** Intelligent drug delivery system based on mesoporous silica nanoparticles. (A) Schematic illustration of the degradation and drug release of the nanozymes with the drug delivery system based on Cu@Fe2C@mSiO2-PEG/LA-R848-ICG-AS1411. [284]. Copyright 2022, American Association for the advancement of science. (B) pH-responsive protein drug delivery system based on dendritic mesoporous silica nanoparticles. [285]. Copyright 2017, Walter de Gruyter GmbH. (C) Mesoporous silica nanoparticles loading plasmid DNA. [286]. Copyright 2011, American Chemical Society.

thereby triggering genomic instability[289,290]. Besides, poor performance was observed in solid tumor cases because DNMT inhibitors (DNMTis) are dependent on DNA participation, making them more effective in actively dividing cells, but not in solid tumors with relatively slow division rates. The instability of DNMTis also causes difficulties in their effective delivery to solid tumors. To date, the efficacy of epigenetic drugs has been primarily applied to hematological cancers[291], partly because solid tumors typically originate from cells with higher degrees of differentiation or even terminal differentiation, whose epigenetic reprogramming ability is low[292]. Furthermore, another important factor limiting efficacy is the intrinsic defects of the drug during treatment, which are attributed to cellular mechanisms affecting drug uptake and metabolism[293]. These drugs showed poor pharmacokinetic properties and difficulties in administration. Due to the chemical instability of the pyrimidine ring in water, DNMTis are not administered orally[294]. Meanwhile, the effect of DNMTis treatment seems to be transient, giving rise to the recovery of DNA methylation signatures after treatment is suspended[295]. Chronic or continuous treatment with DNMTis can engender side effects, including the effects of high-dose 5-azacytidine, which include fetal abnormalities and decreased male fertility[296]. Due to its cytotoxicity and instability, DNMTis cannot be used continuously in patients. To mitigate this effect, stable and safer alternatives to 5-aza-CR and 5-aza-CdR or efficient and precise drug delivery vehicles need to be developed[297,298]. Epigenetic mechanisms also lead to inter- and intratumoral heterogeneity, thereby affecting the efficacy of current epigenetic therapies. Besides, spatiotemporal clonal diversity can exist in the same tumor at the molecular level. Such differences may generate negative responses to therapies that rely on standard care rather than focusing on each individual's unique characteristics.

## 4. Conclusion and outlooks

Cancer is a disease caused by epigenetic disorders of normal cells, and its increasing prevalence is threatening the survival and development of human beings, as well as increasing the burden on happy families, leading to a decline in the quality of life of individuals. Early screening of tumor patients is one of the ways to prevent the deterioration of tumor diseases, and DNA methylation diagnosis has become increasingly important in this regard with the improvement of detection technologies. The development of technologies from bisulfite sequencing to third-generation sequencing, as well as the fine-tuning of detection technologies with different disciplinary fields, has improved the precision and efficiency of the analysis to some extent. As described in the review, each assay has its own scope of application. Therefore, provided that the accuracy of the test is ensured, it is necessary to develop diverse testing platforms to meet the needs of clinicalization. Meanwhile, sophisticated operational requirements and high costs are unavoidable problems with the current commonly used detection technologies. In the future, simplifying the operation steps and reducing the cost of testing will be of positive significance in promoting DNA methylation testing. Regarding the treatment of DNA methylation in the field of oncology, it is currently only used in blood-related tumors, and its application in solid tumors has greater limitations, although the phenomenon of aberrant DNA methylation in tumors is already an indisputable fact. For the application of DNA methylation in tumor therapy, the combination with nanodelivery carriers is an effective way to address the targeting and side effects of DNA methylation drugs. Controlled release of the nanodelivery system can enable DNA methylation drugs to better exert therapeutic effects at the tumor site. In the practical application related to epigenetic drugs, the study of their theory is particularly important. At present, although there is a small amount of mechanism investigation in clinical practice based on DNA methylation, most of it exists in the form of a phenomenal association, especially in the process of diagnosis. There is a lack of in-depth investigation into the mechanisms of DNA methylation, which is the theoretical cornerstone for future improvements in DNA methylation diagnosis and treatment. Exploring the mechanisms of DNA methylation requires great efforts from researchers on the path from phenomena to the identification of molecular biological mechanisms, although it is an extremely taxing process. The in-depth exploration of the DNA methylation mechanism in tumor cells is not only a critical factor in solving the bottleneck of tumor diagnosis and treatment but also an inevitable process to explore the evolution of stem cells in the human body.

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## Conflicts of interests

The authors declare that they have no conflicts of interest.

## Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

## Author contributions

Shikang Liu: conceptualization, writing—original draft, writing—review and editing, and visualization. Youli Yao: visualization. Zhongjun Li: writing—review. Zhiyi Wang: conceptualization, funding acquisition, supervision, and writing—review and editing.

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