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Highlight DNA Methylation Biomarkers in Different Cancer Types for Drug Designing

Samina Gul[1*], **Wenru Tang**[1]

[1] Laboratory of aging and tumor Kunming, University of science and technology, Yunnan, China.

Corresponding author E-mail: samina.gul@cecos.edu.pk

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A B S T R A C T

Recently the study of epigenetics is related to diverse processes such as chromatin structure, tumorigenesis, transcriptional regulation, and genome integrity. Epigenetics defect is one of the new areas of cancer detection. It is a rapidly expanding field of cancer research. Epigenetics is the heritable process that affects gene expression without alteration in DNA sequences. Epigenetics includes three systems DNA methylation, histone modification; Non-coding RNA-associated silencing gene, and chromatin changes. Major epigenetic modifications include DNA methylation patterns that are modified in the cancer cell and used to distinguish a cancer cell from normal and are strongly involved in the physiological control of genome expression. Cancer results from an aberration in genomic DNA including hypo-methylation and hyper-methylation of DNA. Hyper-methylation exhibits the silencing of Tumour suppressor genes. Hypo-methylation activates the transcription of proto-oncogenes, cancer cell metastasis, and genomic instability. Due to the emergence of powerful technologies, the detection of DNA methylation accelerated cancer research. DNA methyltransferases DNMT1 and DNMT3B catalyzed DNA methylation. DNA methylation is associated with loss of gene expression. Different DNA Methylation biomarkers are associated with different cancer types. This review highlights different DNA methylation biomarkers in different types of cancer which are helpful for diagnosis, prognosis, and drug design for curing different types of cancer.

Introduction

All the somatic cell types possess the same genotype and originate from the growth and division of common progenitor cells. Cells became specialized and obtained a variety of functions during the differentiation process by expressing and suppressing different sets of genes. These different s genes contain instruction in two forms: genetics and epigenetics. Genetics instruction is used to manufacture all protein necessary to create a living organism and epigenetic information provide instruction on when, where, and how the genetic information should be used and ensures that which gene is turned on and off at the specific time (Choks, et al., 2004). The epigenetic defect is one of the new areas of cancer detection. It is a rapidly expanding field of cancer research. The epigenetic term is a combination of two words, the Greek prefix “epi” which means upon or over, and “Genetic” which is the science of gene and Heredity, This word was first coined by Conrad Hal Waddington. Epigenetics is a heritable process that affects gene expression without changing the DNA sequences and these changes are transmitted through both

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meiosis and mitosis (Graff, J., et al., 2008). Epigenetic changes are common in human cancer and are reversible and modify the phenotype with no alteration in genotype. Epigenetic changes include histone modification, DNA methylation, and chromatin changes. The covalent addition of a methyl group in the 5-position of cytosine ring in DNA strand refers to DNA methylation. DNA methylation plays a variety of roles in different types of cancer and its profound effect includes transcriptional repression, suppression of detrimental effects of repetitive and parasitic DNA sequences, genome integrity, chromatin structure modulation, X chromosome inactivation, and genome imprinting (Robertson, et al., 2001). DNA methylation regulates and packages the genetic information of the cell and has an important role in many processes including chromatin structure, DNA repair, and genome stability. Many diseases can be easily detected in the light of epigenetics due to methylation and those diseases are Rett syndrome, immunodeficiency originating from abnormal methylation, centrosome instability, and facial abnormalities (PA, et al., 2001). The abnormal methylation includes DNA hypo-methylation and DNA hyper-methylation. DNA hypo-methylation is responsible for overexpression of proto-oncogenes, invasion, and metastasis (M. Szf., et al., 2004). DNA hyper-methylation is responsible for the gene silencing of tumor suppressor genes (Luczak, Michal W., et al., 2006). These DNA methylations which have a profound effect are catalyzed by DNA methyltransferases including DNMT3A, DNMT3B, and DNMT1 (M. Okano., et al., 1998). DNMT1 is considered maintenance and is the most abundant methyltransferases in somatic cells. DNMT3 family are referred to as de novo and they are highly expressed in embryogenesis. Due to the emergence of powerful technologies, the detection of DNA methylation accelerated cancer research. This work focus on DNA methylation biomarker in different cancer types, which can be helpful for diagnosis and drug designing of different cancer types.

Epigenetic Mechanism

The field of epigenetics is very interesting because of relating to various processes such as chromatin structure, genome integrity, transcriptional regulation, and tumorigenesis. Epigenetic changes are responsible for human cancer. For epigenetic changes, the three biochemical mechanisms occur in cells, DNA methylation, histone modification, and the association of non-histone proteins such as polycomb trithorax complexes.

DNA Methylation

The addition of methyl group (-CH3) covalently to the base cytosine(C) in the dinucleotide `5-CpG3` refers to DNA methylation. The base cytosine(C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence refers to the term CpG as shown in Figure 1 (A). In the human genome, most of the CpG dinucleotides are methylated, and un-methylated CpG is clustered together in `CpG` island are not randomly distributed, which facilitates the transcription of a particular gene in the promoter region of many genes. The promoter region of the important gene that has CpG island is mainly un-methylated, in cancer cells methylation of CpG island leads to the silencing of gene expression leading to the hypothesis that DNA methylation plays an important role in the regulation of gene expression shown in Figure 1 (B). The loss of gene expression is also associated with DNA methylation (AP, Bird. 1986). DNA methylation is evolved as a host defense mechanism to inactivate the foreign DNA sequences (B, Hendrich., et al. 1998).

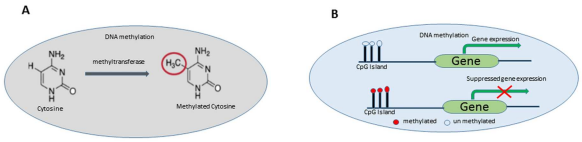

Fig.1- DNA Methylation occurs in presence of methyltransferases enzyme. Figure (a) shows methylation of cytosine in carbon 5 and (b) genes suppression and expression due to DNA methylation

Histone modification

Histone protein is wrapped around the DNA to form a bead-like appearance called a nucleosome, which regulates the accessibility of transcription machinery to DNA (Ghavifekr, F, et al. 2013 & BD, Strahl, et al. 2000). Nucleosome comprised of a tetramer of 2 histone 2A and 2 histones 2B molecules, flanked by H3 and H4 dimers. H3 and H4 N-Terminal tails in t are positively charged in de-acetylated form, leading to a tight chromatin

configuration around the negatively charged DNA. The acetyl group neutralizes the positive charge of lysine residues in N-terminal tails and tight bonds between DNA and histone are loosed and the open chromatin configuration is successfully transcribed (K, Struhl. 1998). The linker histone H1 tied together two consecutive nucleosomes. The linker histone might be altered in cancer cells (CR, Clapie., et al 2009). Post-translation modification includes acetylation, methylation, phosphorylation, and ubiquitination (BD, Strahl, et al. 2000). The methylation and acetylation of specific lysine residues on histone H3 and H4 are most studied. The enzyme which catalyzes these mechanisms are histone acetyltransferases (HAT), HDAC, (HMT) HDMT have identified. The function of these enzymes is either transcriptional activator or repressor complexes. The most studied HAT is associated with complexes such as GCNS, PCAF, MOF, and P300/CBP. These complexes interact and a play very important role in differentiation, development, and cell cycle progression (RT, Utley, et al. 1998). In gene expression regulation Histone methylation also play a major role (T, Jenuwain, Allis CD. 2001). Histone methylation is associated with transcriptional activation depending on specific amino acid-affected methylation of histone H3 Lysine 4 and 36 associated whereas methylation of histone H3 Lysine 9 and 27 is associated with gene silencing. The second group of protein complexes in epigenetics includes the HP1, Polycomb, and trithorax groups. These proteins attach to the DNA methylation or other changes in histone, For example, the polycomb protein complex arrest histone methylation by interacting with DNMT and recruits DNA methylation (T, Jenuwain, Allis CD. 2001). Chromatin structures are affected by a transcription factor and play a role in the heredity of epigenetics changes during cell division (Ghavifekr, Fakhr, et al. 2013). The studying of histone modification in cancer requires a relatively large number of fresh or fresh frozen cells, limiting the study of these in the clinical tissue sample. But in leukemia, some of the data are accumulated (T, Neff, Armstrong SA. 2009). The interaction between different epigenetics mechanism controls the accessibility of genes by the transcription machinery. Epigenetic mechanisms are complete interwind in controlling one another and the function of the target genes in intensifying or attenuating manner in which the activities of these genes can be aligned or nonaligned.

Abnormal DNA methylation and cancer

Cancer is a genetic disease that arises from somatic or series of germline DNA changes. Aberration in genomic DNA methylation results in carcinogenesis. The two different DNA methylation abnormalities are observed in cancer one is global hypo-methylation induces proto-oncogene activation and chromosomal instability other is regional hyper-methylation associated with transcriptional silencing of tumor suppressor gene and inactivating the gene involved in the regulation of the cell cycle, DNA repair, growth signaling, angiogenesis and apoptosis shown in Figure 2.

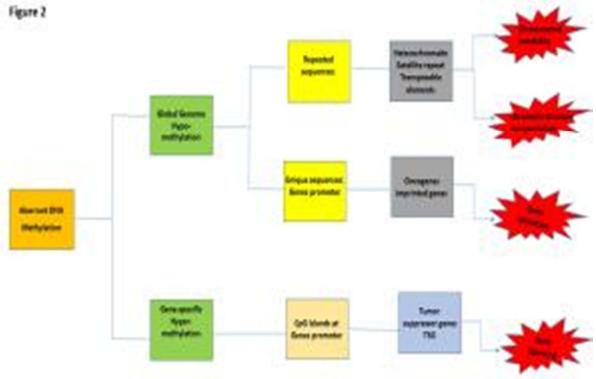

Fig.2 - Aberrant DNA methylation leads to gene silencing, gene activation, chromosomal instability, and chromatin structure reorganization

DNA Hyper-methylation and its role in the inhibition of gene transcription

DNA hyper-methylation occurs at CpG Island and causes gene inactivation which contributes to tumor development shown in figure 3 (PA, Tones, et al. 2007). The inhibition of gene transcription through DNA methylation proposed two mechanisms, one directly inhibits the binding of a transcription factor such as MYC/MYN, AP-2, NFKB, and C- E2F to their binding site within promoter sequence, the binding site of transcription factor CpG dinucleotides sensitive to CpG dinucleotides methylation and second mechanism includes binding of protein-specific for M5CPG dinucleotides to methylated DNA. Methylated DNA enlist M5CPG-binding (MecP) and M5CPG-binding domain (MBD) protein.

MecP is bound to methylated DNA in the form of MecP2 and MecP1 and forms a hurdle that blocks the binding of the transcription factor to promoter sequences. Densely methylated promoter containing more than 10 M5CPG dinucleotides controlled the MecP1 which blocks the transcription of a specific gene. And in the two DNA strand MecP2 bind the M5CPG pair (B, Hendrich, Bird A. 1998). MBD proteins bind to methylated DNA including MBD4, MBD3, MBD2, MBD1, and uncharacterized Kaiso complex. MBD1 inhibits the expression of the gene by blocking the transcription factor interaction with the promoter (N, Fujita, et al. 2000). MBD2 de methylates DNA in vivo and in vitro (H, NGH, Zhang, et al. 1999 & M, SZf., Pakneshan, P, et al. 2004). MBD3 makes an association with MBD2 and targets DNA methylation (Y, Saito, et al. 2002). MBD4 is involved in DNA mismatch pairs (E, Ballestar, et al. 2003 & N, Fujita, et al. 2003). Mecp2, Kaiso complex, MBD1, and MBD2 combine and interact with HADC1 and HADC2 and de-acetylated histones and remodel chromatin structure (N, Fukushima, et al. 2003 & SB, Baylin, Herman, JG. 2000 & R, Brown., et al. 2002 & PM, Das., et al 2004 & PW, Laid. 2005). The repression of transcription genes through DNA hyper-methylation has shown in Figure 3, how the methylated DNA binds m5cpG binding(Mecp5) and m5cpG-binding domain MBD3 protein form hurdle to prevent binding of the transcription factor to a promoter sequence.

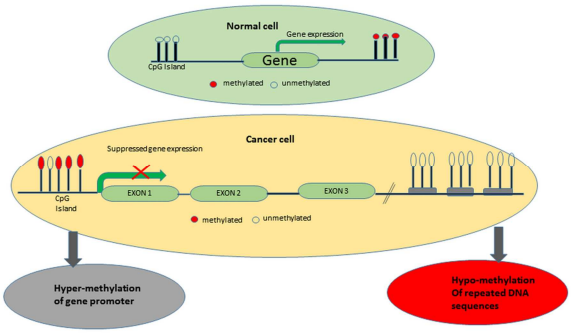

Fig. 3- Gene expression in normal cells and Hypo-methylation, and hyper-methylation in cancer cells

DNA hypomethylation and its role in cancer development

The overall loss of 5-methyl-cytosine is called global hypo-methylation, which occurs in repetitive DNA sequences that comprise approximately half of the human genome shown in Figure 3 (KD, Robertson., et al 2000). Hypo-methylation undergoes tumor progression and metastasis. For example, in blood leukocytes, DNA hypo-methylation increases the bladder cancer risk (Micha, W Ł, et al. 2006). Hypo-methylation overexpress proto-oncogenes which are involved in cancer cell proliferation. DNA hypomethylation induced tumor expressing genes such as PLAU urokinase-type plasminogen activator, (S100A4) Heparanase and calcium-binding protein, Heparanase is released from the malignant cell and degrade heparin sulfate proteoglycan proteases promote metastasis of malignant cell are released during advanced tumor stage. S100A4 increases cell proliferation and motility (Micha, W Ł, et al. 2006). PLAU enzyme is always expressed in prostate and breast cancer (LE, Moore, et al. 2008 & P, Pakneshan, et al. 2004). The most common protease is PLAU, in invasive human breast cancer MDA-231 cells promoter of PLAU is un-methylated (P, Pakneshan, et al. 2005). The promoter of PLAU is methylated in noninvasive human breast cancer MCF-7 cells. IGF2 or insulin-like growth factor2 transcribed from one allele, second is imprinted and transcriptionally inactive, is responsible for cell proliferation in a normal cell, but in tumor cells, hypo-methylation increased bi-allelic expression of IGF2 due to loss of imprinting of the second allele and which stimulate the proliferation and metastasis of cancer cells (P, Pakneshan, et al. 2005). Hypo-methylation of retrotransposons also contributes to carcinogenesis, (LINE) long interspersed nuclear elements belong to retrotransposons, heavily methylated in mammal cells. Hypo-methylation of LINE contributes to genomic instability and tumor progression. LINE hypo-methylation has been observed in chronic lymphatic leukemia and cancer (K, Miyamato, et al. 2005). Global DNA hypomethylation in the malignant cell is still unclear further studies suggested that enzyme involved in methyl transport at cellular level deficiency resulted in DNA hypo-methylation.

DNMT3B contributes to hyper-methylation and hypo-methylation promoting the development, invasion, and metastasis of cancer cells, and is predominantly expressed in malignant as well as human somatic cells, spliced into five different

mRNA isoforms DNMT3B3, DNMT3B4, DNMT3B1, DNMT3B2, DNMT3B5. DNMT3B1 was only present in the ES cell. MCF-7 breast cancer cell line shows an abundant expression of DNMT3B2 and expressed in LD419 fibroblast, t24 bladder cancer cells. The overexpression of DNMT3B4 plays a crucial role in the aberrant expression of cancer-related genes and chromosomal instability (AN, Carnell, et al. 2003). In many types of cancer, the silencing TSG is mainly catalyzed by DNMT1 and DNMT3B (Y, Saito, et al. 2002 & MF, R, et al. 2003).

DNA Methylation biomarkers and different types of cancer:

DNA methylation biomarkers in colorectal cancer

Colorectal cancer is one of the leading causes of death globally, and DNA methylation plays an important role as a diagnostic biomarker in colorectal cancer. Recently SEPT9 has been shown as one of the urinary biomarkers of colorectal cancer. DNA methylation level of SEPT9 is highly elevated in the urine sample of the colorectal cancer patient. DNA methylation can be detected from un-fractioned urine samples of colorectal cancer patients (Ghavifekr, F, et al. 2013). Transcription factor GATA binding protein 4 GATA4 hyper-methylation with a sensitivity of 51–71 % and a specificity of 84–93 % has been identified as one of the biomarkers of colorectal cancer (S. Bach, et al. 2021). NDRG4 is one of the potential biomarkers of colorectal cancer detected from the stool samples of patients and its expression is inactivated by promoter methylation in colorectal cancer (Hassan, A, et al. 2014). IGFBP3 and CD109 DNA methylation are associated with a poor prognosis of colorectal cancer stage II (Yi, JM, et al. 2011). Aberrant DNA methylation leads to colorectal cancer development and progression, and alteration of DNA methylation effect WNT signaling pathways (canonical and non-canonical) both in the gene body and promoter region. CTNNB1, DAAM2, and PRICKLE1 were identified as aberrant methylation new biomarkers of colorectal normal-adenoma-dysplasia sequence progression (Galamb, O, et al. 2016). SFRP1, SFRP2, SOX17, and APC, are identified as DNA methylation biomarker changes in WNT pathways that lead to colorectal carcinogenesis (Galamb, O, et al. 2016). EPAS1 novel potential biomarker in colorectal cancer associated with DNA methylation (Agnieszka, Anna, et al. 2014). *RP1, VIM diagnostic biomarker in colorectal cancer based on DNA hyper-methylation assay (*Afsaneh, M, et al. 2016). *GABRA1 and LAMA2 are the DNA hyper-methylated genes identified for screening and detection of colorectal cancer (*Sunwoo, L, et al. 2012). DNA methylation predicts the genetic basis of colorectal cancer and acts as one of the surrogate markers for prognosis, diagnosis, and therapy of colorectal cancer.

DNA Methylation biomarkers in breast cancer and pancreatic cancer

Breast cancer leading cause of death Worldwide, DNA methylation plays an important role in the detection and screening of breast cancer and acts as a surrogate marker in different types of cancer. Recently MAD12 was identified as a therapeutic biomarker for breast cancer to inhibit DNA methylation in breast cancer (Xiaolong, Xu, et al. 2020). Transcription factor WT1 is identified as hyper-methylated in all breast cancer subtypes and correlated with the poor survival rate of breast cancer patients (Chongyang, Ren, et al. 2021). Recently CT8 was identified as a hypo-methylation oncogene in triple-negative breast cancer (Chen, Chen, et al. 2021). ITGA1, ITGA4, ITGA9 integrin, and Nidogen NID1, NID2 genes were identified as abnormal hyper Methylation in breast cancer (Vladimir, V, et al. 2021). SAT2 and SAT alpha hypo-methylation promote breast cancer (C, J, Rickett., et al. 2018). Pancreatic cancer is the fourth leading cause of death worldwide. DNA methylation biomarker is very effective in the diagnosis of different cancer types. BMP3, FER1L4, and C13orf18 are the DNA methylation biomarker in pancreatic cancer (Massimo, R, et al. 2019). GRAP2, ICAM3, and A2ML1 are potential DNA methylation biomarkers associated with pancreatic cancer (Lingming, K, et al. 2019). DNA methylation biomarkers TGFβ1, TGFβ2, TGFβ3, and TNF are associated with poor survival rates in pancreatic cancer (Brian, Z, H, et al. 2020). CDKN2A, TP53, TTN, KCNJ18, and KRAS are methylation biomarkers in pancreatic cancer (Bin, B, et al. 2020).

Aberrant methylation in SPARC and NPTX2 genes are associated with poor survival rates in pancreatic cancer patients (Nidhi, S, et al. 2020). Hyper methylation in tumor suppressor genes CDO1, TFP12, NPTX2, SFRP1, SFRP2, FOXE1, and PENK are prognostic biomarkers in pancreatic cancer (Cleandra, G, et al. 2020).

DNA Methylation biomarkers in Renal cell carcinoma

DNA methylation act as a promising biomarker for diagnosis, prognosis, and therapeutic response in different types of cancer. Renal carcinoma is one of them. Renal carcinoma is one of the 10 most common cancers of the kidney and shows high resistance to conventional chemotherapy. VHL promoter hypermethylation was identified as highly significant in renal carcinoma (Yang, L, et al. 2016). hypermethylation leads to the silencing of tumor suppressor genes. RASSFIA promoter hypermethylation is associated with renal carcinoma (Hildebrandt, et al. 2010). DNA methylation of silent miRNA-9-1 and miRNA-9-3 in renal carcinoma leads to poor recurrence-free survival (Dubrowinskaja, N, et al. 2014). LAD1, NEFH, and CST6 hypermethylation are associated with a poor prognosis of renal cancer (Pascale, F, et al. 2013). SCL16A3 promoter DNA methylation was identified as a prognostic and diagnostic biomarker in renal cancer (Pai, SI, et al. 2009). DNA methylation is one of the diagnostic biomarkers in renal carcinoma.

DNA Methylation biomarkers in Head, Neck Squamous Carcinoma, prostate cancer

Collective term of malignancies of larynx, pharynx, nose, and oral are known as head and neck squamous carcinoma, aberrant methylation in the promoter region of tumor suppressor genes leads to progression and development of cancer. DAPK, P16, and MGMT promoters abnormal hypermethylation are considered a diagnostic biomarker for head and neck squamous carcinoma. (Chongchang, Zhou, et al. 2018). FAM135B, DDC, ZNF610, and HOXA9, are aberrantly methylated genes associated with the diagnosis and prognosis of head and neck squamous carcinoma (Enchong, Z, et al. 2020). Prostate cancer is one cancer found in men and severe heterogeneous disease and the second leading cause of death in the USA. FOXDI is a DNA methylation biomarker in prostate cancer and is associated with poor prognosis (Shea P. C, et al. 2020). PTSG2 GSTP1, IGFBP7, APC, SFRP2, and IGFBP3, are DNA methylation biomarkers in prostate cancer (Lang, W, et al. 2020). MDM4, VPS53, NUCKS1, PDK1, UHRF1BP1, MCAT, PM20D1, VAMP5, GPR160, LY6G5C DNA methylation biomarkers associated with prostate cancer risk (Irene, M, et al. 2016). AOX1 DNA methylation associated with prostate cancer, CLDN5, and LGALS3 smoking-associated DMR genes methylation is a diagnostic biomarker in prostate cancer (Ken, J, et al. 2010). HOXD3 methylation is associated with prostate cancer (Natasa, v, et al. 2014). PITX2 methylation predicts poor survival in prostate cancer (Montavon, C, et al. 2012).

DNA Methylation biomarkers in Ovarian, Glioblastoma, Lung Cancer

Ovarian cancer is the heterogeneous and sixth leading cause of death-related cancer in women. HOXA9 gene methylation is associated with high-grade ovarian carcinoma (Woloszynska-Read, A, et al. 2007). MAPK, SNGG, and MCJ associated with DNA hypomethylation were up-regulated in ovarian cancer (Christoph, K, et al. 2011). Lung cancer is the second most common cancer in both women and men, and the leading cause of death, in biological processes DNA methylation plays a fundamental role and its major role is associated with cancer. SHOX2 DNA methylation biomarker associated with lung cancer (Shicheng, G, et al. (2014). APC gene is a tumor suppressor that plays an important role in cell migration, adhesion, apoptosis, and transcriptional activation and acts as an antagonist of WNT pathways, APC methylation is a diagnostic biomarker in non-cell small lung cancer (Palmisano, WA, et al. 2000). SAT2 and SAT alpha hypo-methylation promote ovarian cancer (C, Rickett., et al. 2018). Aberrant DNA methylation of P16 and MGMT important diagnosis biomarker in the early detection of lung cancer (Bearzatto, A, et al. 2002). RASSF1A and RAR beta genes tumor-specific methylation are screening biomarkers of early lung cancer detection (Yun-Hua, Z, et al. 2015). CDH13 GENE promoter methylation is a diagnostic biomarker of lung cancer (Yanjun, L, et al. 2020). P16 promoter methylation plays an important role in cancer detection (Yu, S, et al. (2020). Tumor

suppressor of lung cancer miR-132-3p-3p down-regulated via DNA methylation of up-regulated the expression of ZEB2 promote lung cancer (Tingting, Z, et al. 2020). RASSF1 DNA methylation plays a key role in lung cancer screening (Yun-Hua, Z, et al. 2015). Cav-1 DNA methylation is a diagnostic marker of lung cancer (Furong, Y, et al. 2020). GB is a heterogeneous disease and DNA methylation is one of the early events in cancer. MGMT hypermethylation is a diagnostic biomarker in glioblastoma (Jessica, C, et al. 2020). CBLN4, IN4, and RASSF1A promoter methylation are diagnostic biomarkers in glioblastoma (Aleksandra, M, et al. 2020).

Conclusion:

Aberrant DNA methylation includes hypo-methylation and hyper-methylation of DNA play an important role in cancer development and progression. These DNA methylation biomarkers play an important role in diagnosis, and prognosis and are helpful for further studies to design drugs for curing different types of cancer.

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