DNA Methylation Profiling of MYC, SMAD2/3 and DNMT3A in Colorectal Cancer

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Abstract

Epigenetic modifications particularly DNA methylation is a common place and remarkable factor in carcinogenesis transformation. Conspicuously, previous findings have presented a cluster of irregular promoter methylation alterations related with silencing of TSG (tumor suppressor genes), little is accepted regarding their sequential DNA methylation (Hypo and Hyper) modifications during the cancer progression. In this way, fluctuations of DNA methylation of many certain genes especially MYC, SMAD2/3 and DNMT3A have an impressive central key role in many different cancers like colorectal (CRC)one. CRC is distinguished by DNA methylation, which is related

with tumorigenesis and also genomic instability. Importantly, molecular heterogeneity between multiple adenomas in different patients with CRC may show diverse developmental phenotypes for these kinds of tumors. Conclusively, studying of factors which are involved in CRC carcinogenesis, especially the alterations in epigenetic elements, such as DNA methylation besides the RNA remodeling, histone modification, histone acetylation and histone phosphorylation can be influential in order to find new therapeutic and diagnostic biomarkers in this type of malignancy. In this account, we tried to discuss and address the potential significant methylated modifications of these genes and their importance during the development of CRC carcinogenesis.

Keywords: **CRC, DNA methylation, Tumor Suppressor Genes, Genomic instability.**

Introduction

Cancer is a complex cellular mechanism that occurs at least by a mutation of 5 or 6 genes, each mutation alone causes changes in the cell (1, 2). Colorectal cancer is one of the most common types of gastrointestinal cancers that have very high mortality rates (3). The formation of tumors in the rectum, colon, appendix, as well as the extensive and advanced accumulation of genetic and epigenetic changes, alter the natural epithelium of the colon to adenoma and ultimately become as a malignant tumor (4). As a result of genetic and epigenetic changes, colon mucosal changes from normal to cancerous cells. (5). Cancer disrupts the cellular order and this cellular disturbance directly affects the cell cycle and causes a lack of cell differentiation. An increase in the number of cancer patients and also, an increase in the average age of the population has a direct correlation with the increase in cancer in the world. In this regard, the genetic and epigenetic study of different molecular pathways involved in colorectal cancer can be very useful for early diagnosis and treatment (6-10). Many genes are involved in different molecular mechanisms in the carcinogenic pathway, including MYC, SMAD2/3, DNMT3A and many other ones. Here, we want to discuss the performance of MYC, SMAD2/3, DNMT3A alongside with their mechanism in carcinogenesis (11, 12).

Fluctuation of genes

In this way, MYC is transmitted by the avian myelocytomatosis virus and the extent of the gene is widely regulated by viral promoters (13). Some noticeable changes occur in the expression of MYC oncogene and increase the process of cell deformation. MYC oncogene can be produced by

at least 3 different mechanisms. In a number of human tumors, the amount of MYC gene is determined by its natural expression promoters, but the number of copies of this gene is several times as much as the number of copies in the normal human genome (14). Also, in 30% of children's neuroblastoma, a similar gene with c-MYC, called N-MYC, is also widely distributed in malignant tumors **(Figure1).** In both cases, the increase in these genetic copies increases the level of the produced gene. Another point is that MYC family proteins have a very significant effect on cell growth (15). Consequently, when they are present in large quantities, they cause uncontrolled cell proliferation. Myc proto-oncogenes commonly referred to as C-MYC which are distinct from the two N-MYC and L-MYC genes. Human MYC genes are symbolized to as MYC and are seen in a variety of human tumors (16). In addition to genetic and environmental factors, epigenetic factors play a very vital role in carcinogenesis. These factors include histone changes, acetylation, phosphorylation, and DNA methylation (17, 18). DNA methylation, the most essential epigenetic agent, is a common feature in vertebrates, and one of the main epigenetic mechanisms is the control of gene expression (19). Methylation changes can be eliminated or transferred to the next generation without changing the nature of the DNA. Also, CpG methylation is one of the most critical molecular processes in carcinogenesis, and the study of hypermethylation of promoter can create new hopes and achievements in order to achieve molecular diagnostic markers of cancer. In the case of the SMAD gene, it should be stated that the proteins encoded by these genes belonging to the SMAD group of proteins which are similar to those of Drosophila melanogaster genes (Elegans SMAD gene) (20). The SMAD proteins are a signal transducer and transcription modulator that interfaces multiple signaling pathways. These proteins are the main signal transducers for receptors of the transforming growth factor-beta (TGF-β) superfamily, which are critically necessary for regulating cell proliferation, apoptosis and differentiation (21). In response to the signal, TGFβ superfamily ligands bind to a type II receptor, which recruits and phosphorylates a type I receptor. The type I receptor then phosphorylates receptor-regulated SMADs (R-SMADs) which can now bind the coSMAD, SMAD4. R-SMAD/coSMAD complexes accumulate in the nucleus where they act as transcription factors and participate in the regulation of target gene expression (22). Besides SMAD2 which is a protein-encoding gene, major diseases are associated with SMAD2 include colloid, urogenital disease, and many common cancers (23). The pivotal paralog of the SMAD2 is the SMAD3 **(Figure2).** Human immunohistochemistry assessment of SMAD3/SMAD2 phosphorilation and P300 activator showed association with

human glomerulonephritis and renal injury (24). Also, SMAD2 and SMAD4 mutations in the TGF-β -SMAD signaling pathway has been proven in the head and neck carcinoma (25). In addition, the SMAD pathway is also active in scleroderma fibroblast and the level of SMAD2/3 phosphoryl and the site of the SMAD2/3 phosphoryl was increased (26).SMAD2/3/4 heterodimers correspondingly regulate SMDA2/3/4 transcriptional activity. The SMAD3 gene produces a protein that involved in transmitting chemical signals from the cell surface to the nucleus (27). This signaling process begins when TGF-B protein binding to a receptor on the cell surface and activates a group of SMAD proteins such as SMAD2 and SMAD3 (28). These SMAD proteins are form a complex with SMAD4 and then complexes accumulate in the nucleus and binds to specific regions of DNA to control the activity of specific genes. Through the TGF-B signaling pathway, the SMAD2/3 proteins also affect many aspects of cellular processes including growth and division (proliferation), cell movement (migration), and cell death (apoptosis) (29). Another enzyme is the DNA (cytosine-5)-methyltransferase 3A (DNMT3A) that is encoded in humans by the DNMT3A gene (30) **(Figure3).** DNMT3A catalyzes the transfer of methyl groups to specific CpG structures in DNA, a process called DNA methylation (31). DNA methylation plays a role in many cellular functions, such as gene expression regulation, protein and lipid reaction regulation and chemical processing control in signaling of the nervous system that by DNMT3A occurs through methylation during evolution (32). This enzyme can also lead to the formation of more mature cell types in the early cells. In early blood stem cells called hematopoietic stem cells, methylation patterns are generated by the DNMT3A which develops the differentiation to different types of blood cell (33-36).

Induction of epigenetics elements in carcinogenesis and tumorogenesis

Epigenetic elements including DNA methylation, histone modification, histone acetylation, histone phosphorylation and RNAremodeling and the most important one is DNA methylation (37). Additionally, all these elements indicated their remarkable and impressive role in carcinogenesis and also tumorogenesis (38). Unlike changes related to the main DNA sequence, such as mutations, most epigenetic changes are reversible. Naturally, phenomena such as genomic imprinting, inactivation of the X chromosome, and the expression of gene sets that are important in the process of embryonic development are significantly controlled by epigenetic mechanisms (39, 40). In recent decades, studies have shown that epigenetic alteration patterns along with

genetic changes in some genes play an important role in tumorigenesis. These changes include abnormal patterns of g methylation in gene regulatory, histone modification, and alterations in miRNA expression (41). Recent studies suggest that the abnormal pattern of methylation, CpG islands is effective in tumor cell proliferation. So that the increase in the methylation of the regulatory regions of tumor suppressor genes and DNA repair leads to the extinction of these genes and as a result the development of cancer. On the other hand, the reduction of methylation in the regulatory regions of oncogenes increases their expression and leads to the conduction of cells to tumors (42). This mechanism is involved in the development of cancer cells by activating the enzymes involved in the process of cell growth and survival of apoptosis and the cell cycle (43).

Because DNA has an important role in replication and transcription and ultimately cell proliferation, The most important targets are regulatory molecules and anticancer drugs(44).

DNA methylation is regulated by the methyltransferase DNA enzyme. Increasing the expression of DNA methyltransferase appears to be a common feature in a variety of cancers. Methylation patterns are inherited through mitosis. These normal patterns are disrupted in the DNA of the cancer cell; CpG islands are prone to methyltransferase activity and other areas of DNA are hypotensive (45). The hypermethylation profile of CpG islands varies in different genes for each type of cancer. In general, hypermethylation of CpG islands in tumor suppressor genes, genes involved in the cell cycle, DNA repair, carcinogenic metabolism, intercellular interactions, cell death and regression, promotes cancer progression (46).

Performance of DNA methylation in colorectal cancer

Investigating of gene expression in gastrointestinal cancers in order to evaluate their fluctuations, alongside with their epigenetics alterations is of great importance (47-51) (Table 1). Remarkably, the mechanisms underlying CRC pathobiology remain subjects of wide study in the pathogenesis of cancer. Both genetic and epigenetic modifications have resulted in cause of CRC and also the cellular genome that transforms normal glandular epithelium into adenocarcinoma is involved in this process (52, 53). Conspicuously, the evaluation of methylated genes in CRC has also revealed a unique molecular subgroup of colorectal cancers called CpG Island Methylator Phenotype (CIMP) cancers; these tumors have a particularly high frequency of methylated genes. In addition to DNA hypermethylation that often takes places in the promoter region of tumor suppressor genes,

epigenetic regulation of CRC epigenome also includes post-translational histone modifications, primarily histone acetylation and methylation that also play critical roles in regulation of expression of oncogenes and tumor suppressor genes (52, 54, 55). In this way, epigenetics widely alludes to heritable modifications in gene expression that are not mediated by alterations in the DNA sequence. The epigenetic regulation of gene expression happens in normal tissue and it plays an impressive key role in many cellular activities comprising tissue differentiation, embryonic development and also imprinting (56). In 1982, aberrant epigenetic modifications were first explored in CRC. Noticeably, the epigenetic study has indicated an epigenetic landscape comprising of an elaborated array of epigenetic regulatory mechanisms that control gene expression in both tumoral and nontumoral tissues (57, 58). The epigenetic landscape is largely a reflection of agents that ascertain the condensation state of the chromatin, which identifies whether the DNA is reachable to proteins that manage the gene transcription. A relaxed or "open" chromatin state permits for gene transcription, whereas a condensed chromatin state prevents gene transcription (56). Evidently, it is confirmed that the DNA methylation seen in cancer and aging may stem from a small population of cells. Notably, not only are the target sites found partially methylated in normal tissues but are also highly altered in polyps (59), a very early stage in the generation of colon cancer in man. It reveals that during aging, a subpopulation of stem cells in the colon undergoes de novo methylation of target CpG islands, and this presumably generates small patches of tissue that carry an aberrant DNA methylation profile (60-62). This alteration probably induces a state of constitutive heterochromatin, which is not easily reversible. Thus, proliferative cells in the crypt that transform this sign may have this ability in order to skip the polycomb structure itself, but would not be capable of activating the critical differentiation genes, thereby inhibiting these cells from undergoing a transition to epithelium, thus leaving them in a relatively proliferative state. Although this might not be sufficient for generating a tumor, it could very well provide necessary background for cells that have undergone transformation either through prior genetic predisposition or by spontaneous mutation. Meaningly, this particular cells collect and organize DNA methylation during aging and then perform as preferred targets for the transformation process which is protected by the observation that both polyps and normal tissue surrounding the tumor are highly methylated and by the experimental evidence showing that 5-azaC is only capable of preventing the accumulation of intestinal tumors in mice if it is originated from early in life (59, 63, 64). As DNA methylation plays a significant role in CRC formation it

contributes in recognizing the potential diagnostic biomarkers cancer detection(65). Considerably, some gene promoter methylation in the plasma or serum of patients with CRC has been shown great promise as a potential diagnostic indicator of CRC. To date, a lot of hypermethylated genes have been reported in CRC, but only a few have been included in commercial blood-based test. Conclusively, it is required to study in order to find new practical biomarkers progressing prognosis that would contribute researchers and practitioners in order to make a better decision .High-throughput technologies, such as methylation microarrays and next generation sequencing, have helped advance our understanding of epigenetic events at the genomic level (66-68).

Role of DNA methylation of SMAD2/3, MYC and DNMT3A in colorectal cancer

Aberrant de novo methylation of DNA is considered a remarkable mediator of tumorigenesis. The processes that mediate aberrant DNA hyper and hypo-methylation are recently under study. Although certain mechanisms have yet to be identified, it is now clear that DNA methylation is regulated through reciprocal interactions with histones, and that modifications in the post-translational state of histones are closely related with cancer related alterations in DNA methylation (69). The enzymes that mediate DNA methylation, DNMT1, DNTM3a, and DNMT3b, are overexpressed, hyperactive, or misdirected. Increased DNMT expression has been proposed as a mechanism for the increased methylation seen in the promoter region of tumors. Both increased expression and increased function of the DNA methyltransferases have been reported in human cancers, comprising colon cancer, as compared to normal tissues (70). The DNA methyltransferases (DNMTs) catalyze the addition of a methyl group to the 5-cytosine residue of CpG dinucleotides. This family of enzymes comprises DNMT1 that performs as a DNA maintenance methyltransferase, and DNMT3A and DNMT3B that methylate previously unmethylated regions of DNA and are required for genome-wide de novo DNA methylation. Meanwhile it is controversial to attributes the DNMT3A protein expression in human tissue samples and it remains unclear its potential inhibition by classic and novel DNMT inhibitors (71). MYC protein has been implicated both in development through the cell cycle and in differentiation-related regulation of transcription. Additionally, over-expressed MYC protein in dysplastic and tumor cells, accumulating in the cytoplasm and transferring persistently to the nucleus, may modify cellular response to growth factors and abrogate normal growth control mechanisms by controlling cells from escaping from the proliferation cycle (72). Conspicuously,

C-MYC protein may manage its own expression fluctuation via binding process, directly or indirectly, to the C-MYC gene; if this interaction is influenced by DNA methylation (73, 74), there may be a feedback effect between hypomethylation of the third exon of MYC and deregulation of expression. Current research by R.M. Sharrard et al. had indicated that a 34-base pair sequence spanning the CCGG site of the c-MYC third exon exhibits methylation-dependent binding of particular protein species from normal colonic epithelium; dysplastic tissue yields a modified binding pattern (75). Changes in the downstream methylation model may influence the MYC expression through binding of trans-acting agents, either directly or via induction of longer-range conformational alterations. The TGFβ pathway plays a central key role in embryonic development, organ homeostasis, tissue repair, and disease (76, 77). This diversity of tasks is achieved through the intracellular effector SMAD2/3, whose canonical function is to control activity of target genes by interacting with transcriptional regulators (78). In spite of that, a complete description of the factors interacting with SMAD2/3 in any given cell type is still lacking. Bertero et al. recommended that SMAD2/3 could act as a hub coordinating several proteins known to have a role in mRNA processing and alteration, apoptosis, DNA repair, and transcriptional regulation (79). Remarkably, in the therapeutic approach, a probiotic strategy like using gut microbiota is of great importance (80).

The key role of microRNAs in carcinogenesis

Based on the chemical structure of RNA, which is made up of only four flat bases and the nucleotides have a negative charge, it seems that the drug target is not promising; However, RNA molecules can bind to small molecules. The binding of small ligands to the RNA by blocking the macromolecule binding changes the active RNA configuration, induces a sub-configuration on the RNA, and inhibits the RNA catalytic activity, affecting its biological activity. Some herbal anticancer compounds, such as curcumin in turmeric, interact with RNA. More than 80 percent of the genome is actively grouped with RNA transcripts, which are referred to as non-encoding RNAs. This group includes tRNA, rRNA, small nuclear RNA involved in splicing and microRNA (81). MicroRNAs are a type of non-coding non-coding RNA that is fully protected nucleotides during evolution. These molecules are induced by binding to 3' UTR inhibiting translation or induction. Epigenetic factors reduce the expression of microRNAs by over-methylation of gene promoters or histone modifications. Increased expression of microRNAs in cancer cells could be

due to the proliferation and lack of control of a transcription factor or demethylation of CpG islands in gene promoter areas (82). It is not yet clear whether the change in microRNA expression is the result of a pathological state of cancer or whether cancer is the main cause of these changes; However, many microRNAs, especially the two groups of oncogenic micro-RNAs and tumor inhibitors, are abnormally expressed in cancer cells (83). Epigenetic factors reduce the expression of microRNAs by over-methylation of gene promoters or histone modifications. Increased expression of microRNAs in cancer cells could be due to the proliferation and lack of control of a transcription factor or demethylation of CpG islands in gene promoter areas (84).

Conclusion

Cancer is a genetic disease that occurs due to sequential mutations in human genes and also due to genetic and environmental factors. Colorectal cancer is one of the most prevalent lethal forms of cancer in the world (83). Several mechanisms are involved in the development of cancer, which play major roles in altering cellular signaling and cancer formation (84). Oncogenes, tumor suppressor genes, apoptosis genes and restorative genes are among the major factors in cancerous cells (85). These genes are responsible for controlling the differentiation and growth of the cells. Consequently, the mutation in these genes causes the normal process of the cell to become cancerous. Oncogenes are also activated by mutation in the original genes and converted to proto-oncogene. Mutations in tumor suppressor genes also cause abnormal cell division and transforms healthy cells into cancerous ones. Another factor is the programmed cell death (apoptosis), which is the last way of cellular escape from the cancerous process (12). Therefore, study of all factors involved in carcinogenesis, particularly the changes in epigenetic factors, such as DNA methylation, are useful in identifying diagnostic and therapeutic biomarkers in colorectal cancer.

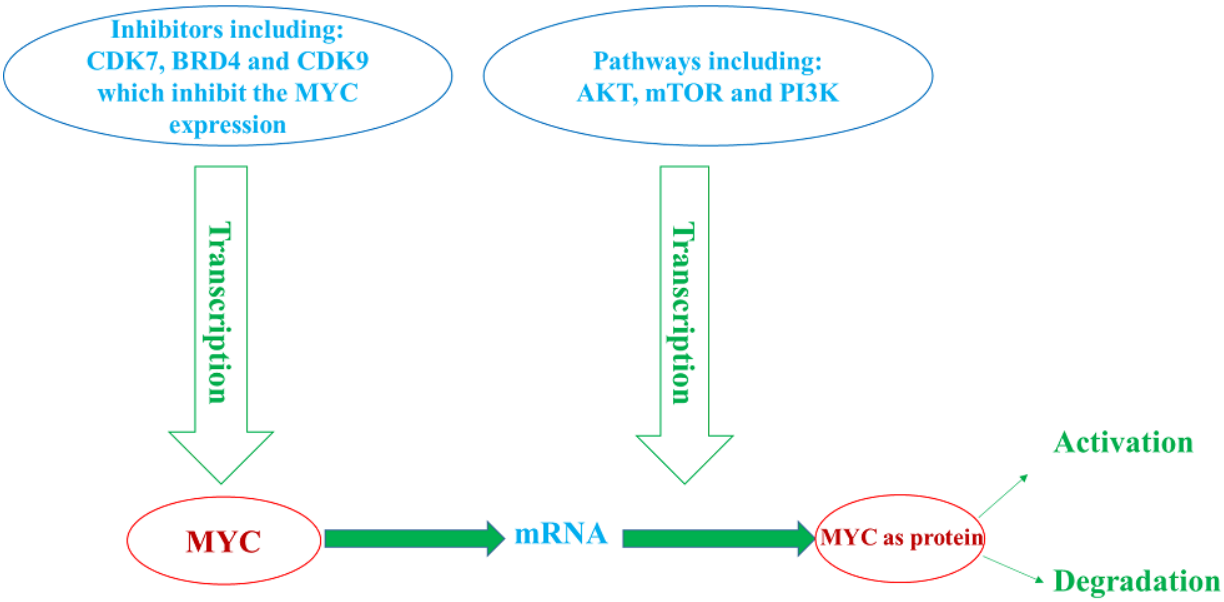

Figure 1. Significance of inhibitors like CDK7, BRD4 and CDK9 in pathways including: AKT, mTOR and PI3K which inhibit the MYC expression.

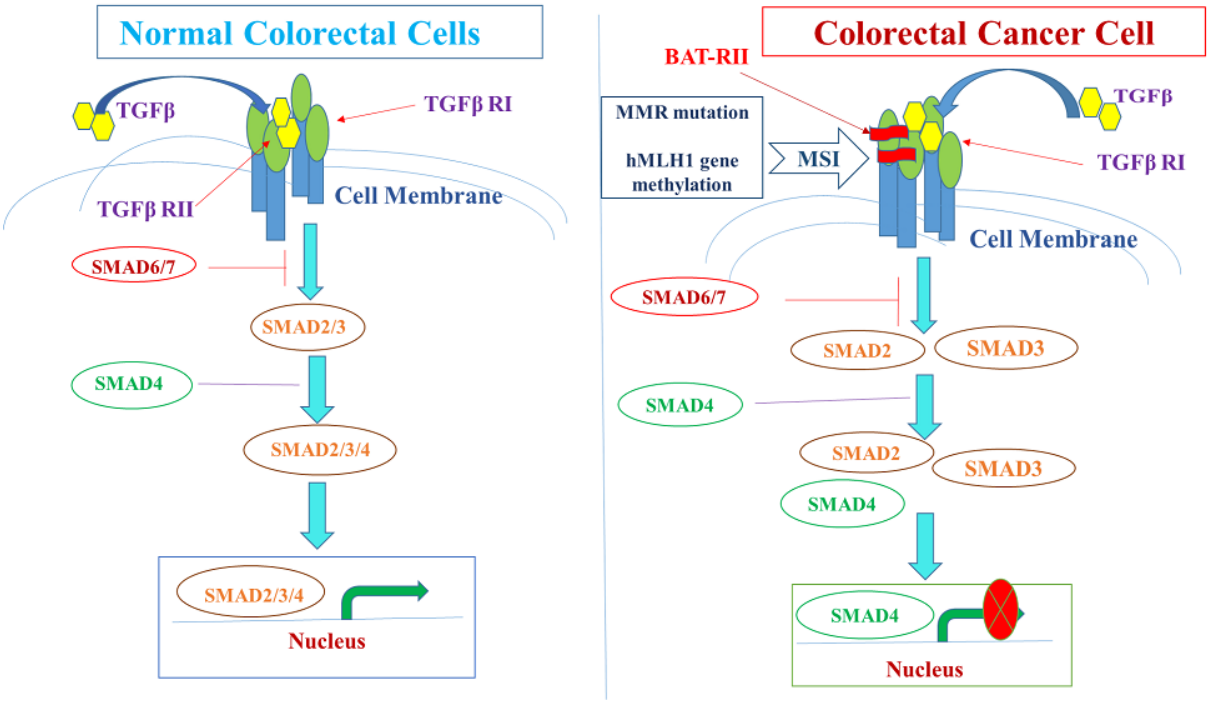

Figure 2. Fluctuations of SMADs (SMAD2/3/4/6 and 7) alongside with other related genes in normal and also colon cancer cells.

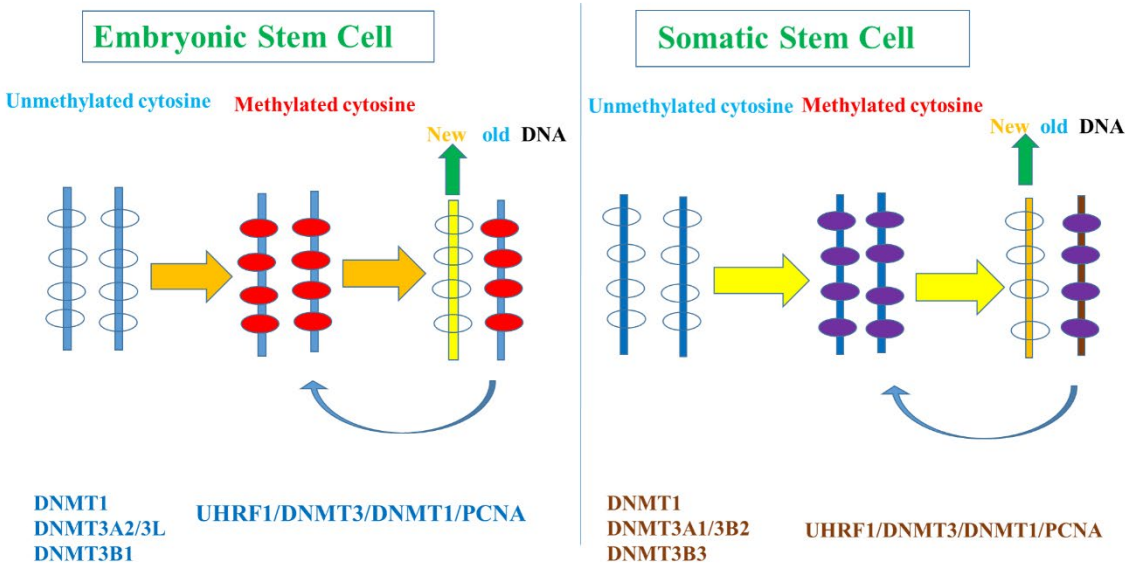

Figure 3. Role of DNMTS in embryonic and somatic Stem Cell.

Table1. Main models of involved genomic uncertainty in colon cancer

Main Disorders	Main Genes	Phenotypic Characteristics	Type of Defect
Sporadic colorectal cancer with mismatch repair deficiency	MLH1 somatic methylation	Colorectal cancer with increased risk of poor differentiation, more commonly located in right colon, less aggressive clinical behavior than tumors without mismatch-repair deficiency	Somatic

Base excision repair defect MYH-associated polyposis	MYH	Development of 15 or more colorectal adenomas with increased risk of colorectal cancer	Germ line
Chromosomal instability- loss of heterozygosity at multiple loci	Loss of heterozygosity at APC, TP53, SMAD4	Characteristic of 80 to 85% of sporadic colorectal cancers, depending on stage	Somatic
CPG island methylator phenotype-methylation target loci	Target loci MLH1,MINT1 MINT2, MINT3	Characteristic of 15% of colorectal cancers, with most showing mismatch repair deficiency from loss of tumor MLH1 expression	Somatic
DNA mismatch repair defects Hereditary nonpolyposis colon cancer	MLH1, MSH2, MSH6 germ line gene mutations	Multiple primary colorectal cancers, accelerated tumor progression and increased risk of endometrial, gastric and urothelial tumors	Germ line

Author contributions

SEN accomplished the data processing, investigated the informatics database, performed the statistical analyses, and wrote the whole manuscript**.** **MGHF**, **MKHP**, **ASH**, **MA**, **VBJ**, **MNM** and **SV** were involved in some sections of the article and evaluated the manuscript ethically. All authors revised the paper comprehensively. All authors read the article comprehensively and confirmed the final version of the manuscript.

Ethical issues

There are no ethical problems for this review article.

Conflict of interests

There is no conflict of interest.

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References

1. Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin D. Cancer incidence and mortality worldwide, international agency for research on cancer. Cancer incidence and mortality worldwide, International agency for research on cancer. 2013:120-63.
2. Samadani AA, Akhavan-Niaki H. Interaction of sonic hedgehog (SHH) pathway with cancer stem cell genes in gastric cancer. Medical Oncology. 2015;32(3):48.
3. Lin S-H, Raju GS, Huff C, Ye Y, Gu J, Chen J-S, et al. The somatic mutation landscape of premalignant colorectal adenoma. Gut. 2018;67(7):1299-305.
4. Terdiman JP, Aust DE, Chang CG, Willenbucher RF, Baretton GB, Waldman FM. High resolution analysis of chromosome 18 alterations in ulcerative colitis-related colorectal cancer. Cancer genetics and cytogenetics. 2002;136(2):129-37.
5. Enderle-Ammour K, Bader M, Ahrens TD, Franke K, Timme S, Csanadi A, et al. Form follows function: Morphological and immunohistological insights into epithelial–mesenchymal transition characteristics of tumor buds. Tumor Biology. 2017;39(5):1010428317705501.
6. Norollahi SE, Hamidian SMT, Kohpar ZK, Azadi R, Rostami P, Vahidi S, et al. The fluctuation of APC gene in WNT signaling with adenine deletion of adenomatous polyposis coli, is associated in colorectal cancer. Journal of Coloproctology. 2019.
7. Babaei K, Shams S, Keymoradzadeh A, Vahidi S, Hamami P, Khaksar R, et al. An insight of microRNAs performance in carcinogenesis and tumorigenesis; an overview of cancer therapy. Life sciences. 2020;240:117077.
8. Akhavan-Niaki H, Samadani AA. Molecular insight in gastric cancer induction: an overview of cancer stemness genes. Cell biochemistry and biophysics. 2014;68(3):463-73.
9. Fattahi S, Langroudi MP, Samadani AA, Nikbakhsh N, Asouri M, Akhavan-Niaki H. Application of unique sequence index (USI) barcode to gene expression profiling in gastric adenocarcinoma. Journal of cell communication and signaling. 2017;11(1):97-104.

10. Kosari-Monfared M, Nikbakhsh N, Fattahi S, Ghadami E, Ranaei M, Taheri H, et al. CTNNBIP1 downregulation is associated with tumor grade and viral infections in gastric adenocarcinoma. Journal of cellular physiology. 2019;234(3):2895-904.
11. Yang X, Zhu F, Yu C, Lu J, Zhang L, Lv Y, et al. N-myc downstream-regulated gene 1 promotes oxaliplatin-triggered apoptosis in colorectal cancer cells via enhancing the ubiquitination of Bcl-2. Oncotarget. 2017;8(29):47709.
12. Wang W, Deng J, Wang Q, Yao Q, Chen W, Tan Y, et al. Synergistic role of Cul1 and c-Myc: Prognostic and predictive biomarkers in colorectal cancer. Oncology reports. 2017;38(1):245-52.
13. Yang T-W, Gao Y-H, Ma S-Y, Wu Q, Li Z-F. Low-grade slightly elevated and polypoid colorectal adenomas display differential β-catenin-TCF/LEF activity, c-Myc, and cyclin D1 expression. World journal of gastroenterology. 2017;23(17):3066.
14. Parang B, Kaz AM, Barrett CW, Short SP, Ning W, Keating CE, et al. BVES regulates c-Myc stability via PP2A and suppresses colitis-induced tumourigenesis. Gut. 2017;66(5):852-62.
15. Mastropasqua F, Marzano F, Valletti A, Aiello I, Di Tullio G, Morgano A, et al. TRIM8 restores p53 tumour suppressor function by blunting N-MYC activity in chemo-resistant tumours. Molecular cancer. 2017;16(1):67.
16. Xie F, Yuan Y, Xie L, Ran P, Xiang X, Huang Q, et al. miRNA-320a inhibits tumor proliferation and invasion by targeting c-Myc in human hepatocellular carcinoma. OncoTargets and therapy. 2017;10:885.
17. Bintu L, Yong J, Antebi YE, McCue K, Kazuki Y, Uno N, et al. Dynamics of epigenetic regulation at the single-cell level. Science. 2016;351(6274):720-4.
18. Tiffon C. The impact of nutrition and environmental epigenetics on human health and disease. International journal of molecular sciences. 2018;19(11):3425.
19. Balch C, Ramapuram JB, Tiwari AK. The epigenomics of embryonic pathway signaling in colorectal cancer. Frontiers in Pharmacology. 2017;8:267.
20. Wotton D, Lo RS, Lee S, Massagué J. A Smad transcriptional corepressor. Cell. 1999;97(1):29-39.
21. Lin H, Yang Q, Wang H, Qi J, Zhang H, Wang H, et al. Involvement of SMAD4, but not of SMAD2, in transforming growth factor-beta1-induced trophoblast expression of matrix metalloproteinase-2. Frontiers in bioscience: a journal and virtual library. 2006;11:637-46.

22. Wrana JL, Attisano L. The smad pathway. Cytokine & growth factor reviews. 2000;11(1-2):5-13.

23. Xu J, Attisano L. Mutations in the tumor suppressors Smad2 and Smad4 inactivate transforming growth factor β signaling by targeting Smads to the ubiquitin–proteasome pathway. Proceedings of the National Academy of Sciences. 2000;97(9):4820-5.

24. Nakao A, Imamura T, Souchelnytskyi S, Kawabata M, Ishisaki A, Oeda E, et al. TGF-β receptor-mediated signalling through Smad2, Smad3 and Smad4. The EMBO journal. 1997;16(17):5353-62.

25. Ottesen JJ, Huse M, Sekedat MD, Muir TW. Semisynthesis of phosphovariants of Smad2 reveals a substrate preference of the activated TβRI kinase. Biochemistry. 2004;43(19):5698-706.

26. Cao Z, Flanders KC, Bertolette D, Lyakh LA, Wurthner JU, Parks WT, et al. Levels of phospho-Smad2/3 are sensors of the interplay between effects of TGF-β and retinoic acid on monocytic and granulocytic differentiation of HL-60 cells. Blood. 2003;101(2):498-507.

27. Hata A, Lo RS, Wotton D, Lagna G, Massague J. Mutations increasing autoinhibition inactivate tumour suppressors Smad2 and Smad4. Nature. 1997;388(6637):82.

28. Stopa M, Anhuf D, Terstegen L, Gatsios P, Gressner AM, Dooley S. Participation of Smad2, Smad3, and Smad4 in Transforming Growth Factor β (TGF-β)-induced Activation of Smad7 THE TGF-β RESPONSE ELEMENT OF THE PROMOTER REQUIRES FUNCTIONAL Smad BINDING ELEMENT AND E-BOX SEQUENCES FOR TRANSCRIPTIONAL REGULATION. Journal of Biological Chemistry. 2000;275(38):29308-17.

29. Xu W, Angelis K, Danielpour D, Haddad MM, Bischof O, Campisi J, et al. Ski acts as a co-repressor with Smad2 and Smad3 to regulate the response to type β transforming growth factor. Proceedings of the National Academy of Sciences. 2000;97(11):5924-9.

30. Huang C, Liu H, Gong X-L, Wu L, Wen B. Expression of DNA methyltransferases and target microRNAs in human tissue samples related to sporadic colorectal cancer. Oncology reports. 2016;36(5):2705-14.

31. Wang C, Jia Z, Ma H, Cao D, Wu X, Wen S, et al. DNA methyltransferase 3a rs1550117 genetic polymorphism predicts poor survival in gastric cancer patients. International journal of clinical and experimental pathology. 2015;8(11):14864.

32. Cacan E, Greer SF, Garnett-Benson C. Radiation-induced modulation of immunogenic genes in tumor cells is regulated by both histone deacetylases and DNA methyltransferases. International journal of oncology. 2015;47(6):2264-75.
33. Zhang W, Xu Y, Ma G, Qi W, Gu H, Jiang P. Genetic polymorphism of DNA methyltransferase 3A rs1550117 A> G and risk of cancer: a meta-analysis. Journal of Investigative Surgery. 2015;28(6):346-53.
34. Norollahi SE, Alipour M, Rashidy-Pour A, Samadani AA, Larijani LV. Regulatory fluctuation of WNT16 gene expression is associated with human gastric adenocarcinoma. Journal of gastrointestinal cancer. 2019;50(1):42-7.
35. Samadani AA, Nikbakhsh N, Taheri H, Shafaee S, Fattahi S, Langroudi MP, et al. cdx1/2 and klf5 expression and epigenetic modulation of sonic hedgehog signaling in gastric adenocarcinoma. Pathology & Oncology Research. 2019;25(3):1215-22.
36. Langroudi MP, Nikbakhsh N, Samadani AA, Fattahi S, Taheri H, Shafaei S, et al. FAT4 hypermethylation and grade dependent downregulation in gastric adenocarcinoma. Journal of cell communication and signaling. 2017;11(1):69-75.
37. Liu X, Luo M, Wu K. Epigenetic interplay of histone modifications and DNA methylation mediated by HDA6. Plant signaling & behavior. 2012;7(6):633-5.
38. Nowacka-Zawisza M, Wiśnik E. DNA methylation and histone modifications as epigenetic regulation in prostate cancer. Oncology reports. 2017;38(5):2587-96.
39. Hongwei S, Tiezhu A, Shanhua P, Chunsheng W. Mammalian DNA methylation and its roles during the induced re-programming of somatic cells. Yi chuan= Hereditas. 2014;36(5):431-8.
40. Juriloff DM, Harris MJ. Hypothesis: the female excess in cranial neural tube defects reflects an epigenetic drag of the inactivating X chromosome on the molecular mechanisms of neural fold elevation. Birth Defects Research Part A: Clinical and Molecular Teratology. 2012;94(10):849-55.
41. Lamba G, Zaidi SK, Luebbers K, Verschraegen C, Stein GS, Rosmarin A. Epigenetic landscape of acute myelogenous leukemia—moving toward personalized medicine. Journal of cellular biochemistry. 2014;115(10):1669-72.
42. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. Nature reviews Drug discovery. 2006;5(1):37-50.

43. Feinberg AP, Tycko B. The history of cancer epigenetics. Nature Reviews Cancer. 2004;4(2):143-53.
44. Hellebrekers DM, Griffioen AW, van Engeland M. Dual targeting of epigenetic therapy in cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2007;1775(1):76-91.
45. Cheung HH, Lee TL, Rennert OM, Chan WY. DNA methylation of cancer genome. Birth Defects Research Part C: Embryo Today: Reviews. 2009;87(4):335-50.
46. Pfeifer GP. Defining driver DNA methylation changes in human cancer. International journal of molecular sciences. 2018;19(4):1166.
47. Samadani AA, Nikbakhsh N, Pilehchian M, Fattahi S, Akhavan-Niaki H. Epigenetic changes of CDX2 in gastric adenocarcinoma. Journal of cell communication and signaling. 2016;10(4):267-72.
48. Ghadami E, Nikbakhsh N, Fattahi S, Kosari-Monfared M, Ranaee M, Taheri H, et al. Epigenetic alterations of CYLD promoter modulate its expression in gastric adenocarcinoma: A footprint of infections. Journal of cellular physiology. 2019;234(4):4115-24.
49. Fattahi S, Nikbakhsh N, Taheri H, Ghadami E, Kosari-Monfared M, Amirbozorgi G, et al. Prevalence of multiple infections and the risk of gastric adenocarcinoma development at earlier age. Diagnostic microbiology and infectious disease. 2018;92(1):62-8.
50. Li J, Jin H, Wang X. Epigenetic biomarkers: potential applications in gastrointestinal cancers. ISRN gastroenterology. 2014;2014.
51. Norollahi SE, Hamidian SMT, Vahidi S, Babaei K, Samadani AA. Modifications of WNT signaling pathway genes including WNT1, KLF5 and WNT16 in colorectal cancer. Gene Reports. 2020:100733.
52. Rodriguez J, Frigola J, Vendrell E, Risques R-A, Fraga MF, Morales C, et al. Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. Cancer research. 2006;66(17):8462-9468.
53. Akhavan-Niaki H, Samadani AA. DNA methylation and cancer development: molecular mechanism. Cell biochemistry and biophysics. 2013;67(2):501-13.
54. Puccini A, Berger MD, Naseem M, Tokunaga R, Battaglin F, Cao S, et al. Colorectal cancer: epigenetic alterations and their clinical implications. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2017;1868(2):439-48.

55. Boot A, Oosting J, van Eendenburg JD, Kuppen PJ, Morreau H, van Wezel T. Methylation associated transcriptional repression of ELOVL5 in novel colorectal cancer cell lines. PloS one. 2017;12(9).
56. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31(1):27-36.
57. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nature Reviews Genetics. 2008;9(6):465.
58. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128(4):683-92.
59. Nejman D, Straussman R, Steinfeld I, Ruvolo M, Roberts D, Yakhini Z, et al. Molecular rules governing de novo methylation in cancer. Cancer research. 2014;74(5):1475-83.
60. Norollahi SE, Mansour-Ghanaei F, Joukar F, Ghadarjani S, Mojtahedi K, Nejad KG, et al. Therapeutic approach of Cancer stem cells (CSCs) in gastric adenocarcinoma; DNA methyltransferases enzymes in cancer targeted therapy. Biomedicine & Pharmacotherapy. 2019;115:108958.
61. Samadani AA, Norollahi SE, Rashidy-Pour A, Mansour-Ghanaei F, Nemati S, Joukar F, et al. Cancer signaling pathways with a therapeutic approach: An overview in epigenetic regulations of cancer stem cells. Biomedicine & Pharmacotherapy. 2018;108:590-9.
62. Samadani AA, Noroollahi SE, Mansour-Ghanaei F, Rashidy-Pour A, Joukar F, Bandegi AR. Fluctuations of epigenetic regulations in human gastric Adenocarcinoma: How does it affect? Biomedicine & Pharmacotherapy. 2019;109:144-56.
63. Belshaw NJ, Pal N, Tapp HS, Dainty JR, Lewis MP, Williams MR, et al. Patterns of DNA methylation in individual colonic crypts reveal aging and cancer-related field defects in the morphologically normal mucosa. Carcinogenesis. 2010;31(6):1158-63.
64. Klutstein M, Nejman D, Greenfield R, Cedar H. DNA methylation in cancer and aging. Cancer research. 2016;76(12):3446-50.
65. Sameer AS, Nissar S. Epigenetics in diagnosis of colorectal cancer. Molecular biology research communications. 2016;5(1):49.
66. Ma Z, Williams M, Cheng YY, Leung WK. Roles of Methylated DNA Biomarkers in Patients with Colorectal Cancer. Disease Markers. 2019;2019.
67. deVos T, Molnar B. Screening for colorectal cancer based on the promoter methylation status of the septin 9 gene in plasma cell free DNA. J Clin Epigenetics. 2017;3:1-6.

68. Danese E, Montagnana M. Epigenetics of colorectal cancer: Emerging circulating diagnostic and prognostic biomarkers. Annals of translational medicine. 2017;5(13).
69. McCabe MT, Lee EK, Vertino PM. A multifactorial signature of DNA sequence and polycomb binding predicts aberrant CpG island methylation. Cancer research. 2009;69(1):282-91.
70. Lao VV, Grady WM. Epigenetics and colorectal cancer. Nature reviews Gastroenterology & hepatology. 2011;8(12):686.
71. Yang SX, Nguyen D, Rubinstein L, Chen AP, Doroshow JH. Abstract B12: DNA methyltransferase 3A expression in cancer cells following treatment with DNMT inhibitors and in human solid tumors. AACR; 2015.
72. Freytag SO. Enforced expression of the c-myc oncogene inhibits cell differentiation by precluding entry into a distinct predifferentiation state in G0/G1. Molecular and cellular biology. 1988;8(4):1614-24.
73. Prendergast GC, Ziff EB. Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. Science. 1991;251(4990):186-9.
74. Elbadawy M, Usui T, Yamawaki H, Sasaki K. Emerging Roles of C-Myc in Cancer Stem Cell-Related Signaling and Resistance to Cancer Chemotherapy: A Potential Therapeutic Target Against Colorectal Cancer. International journal of molecular sciences. 2019;20(9):2340.
75. Sharrard R, Royds J, Rogers S, Shorthouse A. Patterns of methylation of the c-myc gene in human colorectal cancer progression. British journal of cancer. 1992;65(5):667.
76. Wu MY, Hill CS. TGF-β superfamily signaling in embryonic development and homeostasis. Developmental cell. 2009;16(3):329-43.
77. Oshimori N, Fuchs E. The harmonies played by TGF-β in stem cell biology. Cell stem cell. 2012;11(6):751-64.
78. Gaarenstroom T, Hill CS, editors. TGF-β signaling to chromatin: how Smads regulate transcription during self-renewal and differentiation. Seminars in cell & developmental biology; 2014: Elsevier.
79. Bertero A, Brown S, Madrigal P, Osnato A, Ortmann D, Yiangou L, et al. The SMAD2/3 interactome reveals that TGFβ controls m 6 A mRNA methylation in pluripotency. Nature. 2018;555(7695):256.

80. Vieira AT, Fukumori C, Ferreira CM. New insights into therapeutic strategies for gut microbiota modulation in inflammatory diseases. Clinical & translational immunology. 2016;5(6):e87.

81. Sana J, Faltejskova P, Svoboda M, Slaby O. Novel classes of non-coding RNAs and cancer. Journal of translational medicine. 2012;10(1):103.

82. Ruan K, Fang X, Ouyang G. MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer letters. 2009;285(2):116-26.

83. Esteller M. Non-coding RNAs in human disease. Nature reviews genetics. 2011;12(12):861.

84. Cho WC. MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. The international journal of biochemistry & cell biology. 2010;42(8):1273-81.

85. Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, et al. Colorectal cancer screening: recommendations for physicians and patients from the US Multi-Society Task Force on Colorectal Cancer. The American journal of gastroenterology. 2017;112(7):1016.

86. Lee J-Y, Kong G. Roles and epigenetic regulation of epithelial–mesenchymal transition and its transcription factors in cancer initiation and progression. Cellular and molecular life sciences. 2016;73(24):4643-60.

87. Karki R, Malireddi RS, Zhu Q, Kanneganti T-D. NLRC3 regulates cellular proliferation and apoptosis to attenuate the development of colorectal cancer. Cell Cycle. 2017;16(13):1243-51.