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Relationship between DNA methylation mechanism and Sonic Hedgehog family (SSH) genes in carcinogenesis and tumorigenesis of gastrointestinal cancers

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Abstract

Gastrointestinal cancers are among the most serious cancers. In addition to environmental factors, genetic and epigenetic factors also play a key role in the development of gastrointestinal cancers. Since many molecular pathways are involved in the development of this type of cancer, the study of the function of molecular mechanisms involved in carcinogenesis and tumorigenesis and their relationship with genes involved in this malignancy in different molecular pathways to identify biomarkers used in early detection it is of great importance. The role of the Sonic Hedgehog signaling pathway in maintaining and controlling stem cell growth and its association with genes involved in gastrointestinal cancers has been reported. In this study, the importance and relationship between the DNA methylation mechanism and genes of the Sonic Hedgehog family in carcinogenesis and tumorigenesis of gastrointestinal cancers will be investigated.

Keywords: Sonic Hedgehog, Carcinogenesis, Tumorigenesis, Gastrointestinal cancers

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Introduction

Role of Epigenetics in cancer progression

Gastrointestinal cancers include malignancies in the esophagus, stomach, liver, bile ducts, gallbladder, pancreas, colon and small intestine. Due to the late detection of cancers, the resulting mortality is very high. Although the incidence and survival rate vary according to the organs involved, early diagnosis and prognosis are very effective in treating and reducing mortality (1).

Epigenetic changes, including DNA methylation, histone alterations, histone acetylation, chromatin alteration, and microRNA expression, regulate gene expression through mechanisms other than genomic DNA sequencing and are associated with human cancer. Meanwhile, DNA methylation is an essential epigenetic process to modulate gene expression (2-4). In DNA methylation, a methyl group (CH3-) is added to the carbon of 5 cytosine rings in CpG dinucleotides (5). This reaction is catalyzed by enzymes called DNA methyltransferases (6). CpG dinucleotides are not uniformly distributed throughout the human genome. Areas of the genome that are rich in CpG dinucleotides are known as CpG islets. Changes in DNA methylation in human cancers include hypermethylation and hypomethylation. Epigenetic changes, including DNA methylation, are reversible and are therefore used as therapeutic targets for cancer (7). It should be noted that these changes are inherited through cell division. DNA methylation as an essential part of a normal organism is important in the process of cell growth and differentiation and modulation of gene expression patterns in cells (8). DNA methylation is involved in modulating the activity of genes without changing their sequence and has been suggested as the most important factor in oncogenic evolution (9). Epigenetic regulation of gene expression is also important as an essential method in the pathogenesis of many malignancies, including gastrointestinal cancers. Many studies have identified DNA methylation levels as biomarkers in the diagnosis, risk assessment, and prognosis for gastrointestinal cancers (10).

Hatchback proteins are secretory signaling proteins that were first observed in Drosophila melanogaster along with many compounds of the signal transduction mechanism. These proteins are significantly hydrophobic so that after secretion, they are dispersed in the tissue and are involved in embryonic development (11).

Among the genes of the Hedgehog family, most studies have been done on the genes of the Sonic Hedgehog pathway, mainly due to its dominant pattern in different tissues and the application of experiments related to the Sonic Hedgehog protein for other homologs of the Hedgehog family. Significantly, one of the most important molecular pathways involved in cancer is the sonic signaling pathway. HRHC mRNA and sonic protein expression have been reported in the adult gastrointestinal tract, and gastric wall cells (12). Sonic Hedgehog is a 45 kDa protein encoded by the Sonic Hedgehog genes. Sonic Hedgehog plays an important role in regulating vertebrate organogeneses, such as organic growth and brain organization. In addition, it controls the cell division of adult stem cells and is involved in the development of some types of cancer (13). Sonic Hedgehog signaling activates proliferation in adult tissues during differentiation. In addition, there is abnormal activation of sonic signaling in various cancers, especially gastric, colon, skin, brain, liver, gallbladder, pancreas, breast, lung, prostate and blood malignancies (14). This study aimed to investigate the relationship between the DNA methylation mechanism and genes of the Sonic Hedgehog (SSH) family in carcinogenesis and tumorigenesis of gastrointestinal cancers.

A study was conducted in 2015 to investigate the interactions of the Sonic Hedgehog signaling pathway with cancer stem cell genes in gastric cancer. This study has shown that DNA methylation is one of the most important epigenetic changes in gastric cancer, and identifying the signaling mechanism as well as methylation of some genes involved in gastric cancer can help improve treatment strategies. Relatively, many changes in the methylation of genes in stem cells have been reported in a variety of tumors, some of which play a key role in tumor formation. KLF5, CDX1 / 2, WNT1 and FEM1A are significant genes in gastric cancer, although many studies and studies have shown that Sonic Hedgehog and its protein expression are associated with gastric cancer. Relatively, changes in these genes cause many inflammatory cancers, such as rhabdomyosarcoma and various types of

gastrointestinal cancers. Therefore, it was concluded that research and studies related to the methylation and expression of these genes as well as the study of molecular signaling in gastric cancer can be of significant importance in controlling and treating this serious problem (15).

In a review study, the effect of changing the oscillations of some important genes in the Sonic Hedgehog signaling pathway in gastric adenocarcinoma was investigated. The results of this study showed that the WNT and SHH signaling pathways play a key role in controlling the growth and maintenance of stem cells as well as the relationship of these signaling pathways with CDX1 and CDX2 genes in gastric cancer. In this study, the relationship between the role of epigenetic factors, especially DNA methylation in gastric cancer with molecular pathways such as SHH, NOTCH and WNT in the carcinogenic phenomenon was investigated and interpreted (16).

In another research to investigate the expression of CDX1 / 2 and KLF5 genes and epigenetic changes in the Sonic Hedgehog signaling pathway in gastric adenoma-carcinoma. Changes in DNA methylation of CDX1, KLF5 and CDX2 genes were evaluated by evaluating the expression of these genes in gastric cancer tissues and tumor peripheral tissues using specific methylation sequence and Real-time PCR Taq man assay, respectively. The results showed a significant decrease in the expression of CDX1 and KLF5 genes and an increase in the expression of CDX2 gene, which is involved in the Sonic Hedgehog signaling pathway. While the results of methylation regulation in CpG regions showed significant fluctuations that were not significantly different in most areas. The formation of metastatic lymph nodes in patients was also significantly associated with increased CDX2 gene expression. The expression of these genes can be considered as a biomarker of cancer in future studies if the methylation of the studied genes can not be considered the main mechanism of gastric cancer (17).

Jeng et al. reviewed the role of the Sonic Hedgehog signaling pathway in organogenesis, tumors, and the tumor microenvironment was investigated. The role and abnormal activation of Sonic Hedgehog signaling in skin, brain, liver, gallbladder, pancreas, stomach, colon, breast, lung, prostate and blood cancers have been interpreted in this study. The results of this study showed that the Sonic Hedgehog signaling pathway plays an important role in cancer, organogenesis and tumor microenvironment of some organs. The combined use of Sonic Hedgehog signaling pathway inhibitors, chemotherapy, radiation therapy and immunotherapy can play a key role in targeting cancer stem cells. In addition, a better understanding of these mechanisms can help to properly target the Sonic Hedgehog signaling pathway against cancer (12).

In Fu et al. study, the regulatory mechanism of ligand expression of this pathway in colorectal cancer was clarified. The findings showed an increase in the expression of Sonic Hedgehog proteins in colorectal cancer compared with hyperplastic polyps and colon adenoma. Consequently, in colorectal cancer, hypothyroidism of Sonic Hedgehog signaling leads to activation of the Sonic Hedgehog pathway associated with Sonic Hedgehog signaling (18).

Extracellular signaling

Membrane receptors and co-receptors are used by both the Hh and Wnt signaling pathways to detect ligand and transmit the signal to the nucleus. Co-receptors can either bind directly ligands or alter the binding of ligands to other receptors. Cells can release proteins that sequester ligands, inhibiting pathway activation, in addition to membrane-attached receptors. Patched-1 (PTCH1) is the main receptor for Hh ligands, which inhibits pathway activation in the absence of Hh ligand. When PTCH1 is internalized and destroyed in the presence of ligand, smoothened (SMO) is translocated to the cell surface on the primary cilium. The glioma-associated oncogene (GLI) transcription factors are then activated by SMO (19). Our several PTCH1 isoforms, PTCH-1A, PTCH–1 B, and PTCH-1C, have been found. The promoters for PTCH-1, PTCH-1A, and PTCH–1 B each have a separate CpG island, while the promoter for PTCH-1C has a distinct CpG island. The GLI binding sites of the PTCH1 gene are found in the regulators of isoforms PTCH–1 B and PTCH-1C, making them Hh signaling pathway targets. Increased expression of PTCH–1 B and PTCH-1C isoforms occurs when the Hh cascade is activated, leading to a negative feedback mechanism. The PTCH1 gene's lack of activity reduces SMO inhibition, resulting in ligand-

independent activation of the Hh pathway. PTCH1 is a tumor suppressor because of its critical function in regulating the Hh pathway and thus directly inhibiting unregulated cell proliferation. It is frequently altered in Hh pathway-driven malignancies. Although the early study did not find epigenetic silencing of the PTCH1 gene in MB and BCC, even when near-absent PTCH1 mRNA expression was assessed, epigenetic suppression of the PTCH1 gene would be a plausible oncogenic occurrence (20). Unfortunately, because of the high CG content of its promoter, this research focused on the PTCH–1 B isoform. The recent investigation into the methylation status of the PTCH1 gene discovered that the PTCH-1C isoform's promoter was methylated. However, one investigation employing a more sensitive technique for identifying methylation found low levels of methylation in the PTCH–1 B promoter in BCC. Others have reported reactivation of PTCH1 expression in MB after a simultaneous decrease of methyltransferases and histone deacetylases (HDAC), indirectly showing epigenetic regulation of the PTCH1 gene, in recent experiments measuring PTCH1 promoter methylation (21).

Tumor formation is aided by activating mutations in the SMO gene, but the epigenetic contributions to abnormal SMO activity are less well understood. The promoter region of SMO is hypermethylated in various MB cell lines, but this does not appear to have a substantial impact on SMO transcriptional activity. Furthermore, numerous miRs regulate SMO expression epigenetically in healthy tissue. The loss of these miRs occurs as a result of the MB-associated deletion of chromosome 17p, resulting in SMO overexpression. SMO expression in BCC is influenced by epigenetic alterations that have yet to be discovered (22).

Many other proteins aid in Hh ligand binding, sequestering, and signal transduction, in addition to primary controllers of the Hh pathway, PTCH1, and SMO. GAS1, CDON, BOC cell-adhesion involved, oncogene connected (BOC), and LDL receptor-related protein 2 (LRP2) all work together to increase HH ligand-PTCH1 interactions. CDON also acts as a dependency receptor, causing apoptosis in the lack of Hh ligand. The number of epigenetic studies conducted on these coreceptors is very minimal. GAS1 binds to miR-34a, and this miR is downregulated in MB cells. In MB, the BOC gene is hypomethylated downstream of its TSS, which is linked to a significant level of expression. Changes in the epigenetic regulation of CDON and LRP2 have yet to be discovered. HH target cells can express Hedgehog interacting protein in combination to signal to boost proteins (HHIP). This protein binds to HH ligands with a high affinity, preventing them from signaling. In MB cell lines, the promoter of HHIP is methylated, leading to decreased expression. Conversely, methylation is significantly less common in primary patient-derived tumor tissues, implying that there are additional HHIP regulatory mechanisms in MB (23).

E3 ubiquitin ligases ring finger protein 43 and zinc and ring finger 3 (RNF43/ZNRF3) regulate extracellular Wnt signaling by targeting FZD and LRP6 for degradation. RNF43/ZNRF3 forms a complex with leucine-rich repeat-containing G protein-coupled receptors 4, 5, or 6 (LGR4/5/6), resulting in the accumulation of Wnt receptors on the cell membrane in the presence of R-spondin (RSPO1) (24). MiR-550-5p silences RNF43 epigenetically in CRC by targeting its 3′-UTR. SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) binds to the promoter of miR-550-5p and silences it. SMARCA4 is dysregulated in CRC and BC, which causes RNF43 to be downregulated as well. In CRC2, SMARCA4 decreased expression results in an increase in metastasis (25).

Across both CRC and BC, the promoter regions of four of the five close relatives (SFRP1, SFRP2, SFRP4, and SFRP5) are commonly hypermethylated, and this corresponds with expression. Furthermore, methylation levels rise with the tumor stage (26). Wnt-receiving cells express cadherin proteins in addition to Wnt receptors and co-receptors. Cadherins increase cell-cell adhesion, allowing tissue architecture to be maintained while limiting metastasis and proliferation. CTNNB1 is also localized to the cell surface by numerous cadherin types, which inhibits Wnt signaling. As a result, cadherin loss of function may contribute to Wnt-mediated carcinogenesis on multiple levels. Indeed, miRs frequently hypermethylated or downregulate promoter regions of Wnt-related cadherin genes in BC and CRC (27).

Intracellular signaling

Membrane-bound receptors detect Hh and Wnt signals, which are then sent to the nucleus via various intracellular proteins. The epigenetic processes that influence these pathway components are listed in Table 1. The Hh pathway's intracellular signal transduction is dependent on a balance shift between the activator and repressor forms of the GLI transcription factor family members GLI1, 2, and 3. DNA-binding sites and a C-terminal activation domain are found in all three members. GLI2 and GLI3 also have a repression domain at the N-terminus.

Table 1. Mechanisms of epigenetic regulation involving and Wnt pathway in cancer..

Protein	Tumor type and expression fluctuation	Mechanism
SHH	GC ↑	Promoter hypomethylation
	BC ↓	Histone modification, promoter hypomethylation and hypermethylation
SMO	MB↑	miR downregulation, Promoter hypomethylation
	NCCLC ↓	miR downregulation
	GC ↓	miR downregulation
BOC	MB↑	Promoter hypomethylation
GLI1	MB↑	miR downregulation
GLI2	**BC ↑**	Histone modifications
	GC ↑	miR downregulation
	MB↑	miR downregulation/ upregulation
	BC ↑	miR upregulation
	GC ↑	miR downregulation and lncRNA upregulation
WNT2	CRC ↑	Histone modifications
WNT5A, WNT9B, WNT10A	CRC ↓	Promoter hypermethylation
LRP5/6	BC, GC, PDAC, HCC ↑	miR downregulation
FZD	BC, GC, PDAC, HCC ↑	miR downregulation
DKK	CRC ↓	Promoter hypermethylation
Cadherin	CRC ↓	Promoter hypermethylation
CTNNB1	CRC↑	No promoter methylation change
APC	CRC ↓	Promoter hypermethylation
AXIN	CRC ↓	miR downregulation

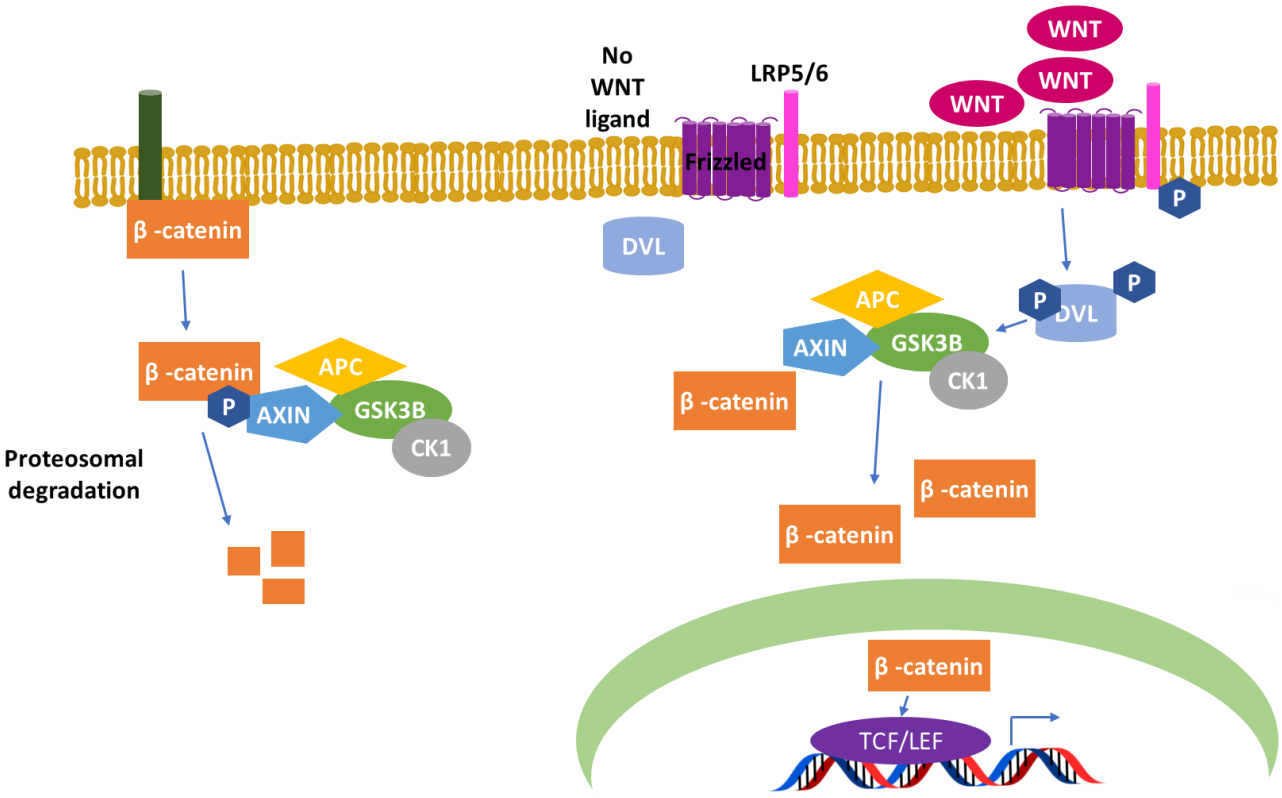
In the absence of Hh signaling, various proteins phosphorylate the C-terminal activation domains of GLI2 and GLI3, which are then ubiquitinated by -transducin repeat-containing protein (TrCP), resulting in partial proteasomal destruction of GLI2 and 3. This is aided by Kinesin family member 7 (KIF7). The DNA-binding sites and the N-terminal repressive domain of the residual repressor proteins translocate to the nucleus, where they block transcription of Hh target genes. However, due to slight variations between the so-called processor determinant domains of both genes, GLI2 processing is substantially less effective than GLI3 processing. As a result, GLI3R is the Hh pathway's primary inhibitor. Even though the GLI proteins are critical for the totality of the biological effects of the Hh pathway, little is known about their epigenetic control. Several miRs have been identified as controlling one or more GLI family members. MiRs 218 and 324-5p, which are also deleted in MB, inhibit GLI1 (28). Although GLI3 is the primary repressor of the GLI proteins, hypermethylation of its promoter in MB appears to have no effect. In addition, the loss of miR-378, which regulates GLI3 in normal tissue, causes GLI3 to be overexpressed in BCC (29).

So yet, no evidence of epigenetic regulation of the proteins that phosphorylate and ubiquitinate GLI2 and GLI3 has been found in MB or BCC.

Suppressor of Fused (SUFU), a negative regulator of Hedgehog signaling, regulates the intracellular Hh pathway in contrast to GLI processing. Unprocessed GLI proteins form a combination with SUFU, which prevents them from accessing the nucleus. Even though SUFU is a potent potential potent inhibitor, there are few investigations on its epigenetic regulation in MB and BCC. In MB, abnormal promoter methylation does not appear to influence SUFU. SUFU, on the other hand, is epigenetically downregulated in BCC by miR-455-5p. As a consequence of the epigenetic

suppression of SUFU, the level of GLI protein may rise (30). APC gene mutations are detected in 80% of sporadic CRCs, and APC loss is assumed to be the primary cause of CRC initiation. In particular, regardless of APC mutation status, APC promoter hypermethylation is common in CRC. Mutations in the APC gene frequently result in truncated proteins with only partial performance. Epigenetic silencing of the defective gene can further diminish mutant APC expression and contribute to the growth of CRC tumors (31). Hypermethylation of the APC promoter is also seen in BC, contributing significantly to the decrease of APC expression. Hypermethylation of the APC promoter is more common in late-stage BC (32). The GSK3 B promoter is significantly methylated in CRC, but it is only found in a small percentage of cases and is unrelated to expression. In contrast, miRs 224 and 1229, which are increased in CRC and BC, respectively, directly target the GSK3 B promoter (33). Because the YAP1/WWTR1 proteins were just recently connected to the Wnt signaling pathway, their significance in Wnt-related cancer is yet unknown. A dual role for YAP1/WWTR1 has been suggested: in addition to directing TrCP to the destruction complex, free YAP1/WWTR1 can act as a positive Wnt signaling transcriptional regulator. Even though the function of YAP1/WWTR1 in Wnt signaling is becoming more well-known, there has been little investigation on its epigenetic regulation. MiR-506, which is elevated in both CRC and BC, targets YAP1, while miR-125a, which is elevated in BC, targets WWTR1 (34). Wnt target gene transcription is suppressed in the absence of nuclear CTNNB1 by t-cell factor/lymphoid enhancer-binding factor 1 (TCF/LEF1) family members and transducing-like enhancer protein recruitment by a reactionary related protein TCF/LEF1 family members and transducing-like stimulator protein (TLE). FZD recruits Dishevelled segment polarity proteins (DVL) to the cell membrane in the presence of Wnt ligands, where they are phosphorylated and polymerized. After DVL binds AXIN, the destruction complex is effectively translocated to the cell membrane. GSK3 B and CSNK1A1 can then phosphorylate LRP co-receptors, causing additional AXIN to be recruited to the cell membrane and creating a positive feedback mechanism. Wnt signaling separates YAP1/WWTR1 from the destruction complex, and therefore TrCP (35) (Figure1).

Figure 1. Wnt ligand signaling and production.



Members of the TCF/LEF1 family are powerful oncogenes that play a role in Wnt signaling and target gene activation. As a result, during carcinogenesis, epigenetic regulation of these genes may be lost. In CRC, TCF homologs' promoters (TCF7L1 and TCF7L2) are frequently hypomethylated, and TCF transcription regulation by miR-29 is also lost. The promoter regions P1 and P2 of LEF1 are divided into two halves. Wnt signaling upregulates P1 transcription since it is a Wnt target. When P2 is transcribed, a dominant-negative LEF1 isoform (dnLEF1) is produced, which inhibits cell growth. By producing H3K9me3 repressive epigenetic marks, the YY1 transcription factor (YY1) epigenetically silences P2. In BC, YY1 is increased, while the repressive dnLEF1 isoform is epigenetically silenced. MiR-34, which is deleted in both CRC and BC due to p53 loss, is also a target for LEF1 (36, 37).

Conclusions

Given current breakthroughs in epigenetic control of the Hh and Wnt pathways, there is currently no therapeutically effective therapy for epigenetically regulated Hh and Wnt-driven malignancy. This is partly because signaling mechanisms for these pathways have only lately been found or are yet unknown. For example, little is understood about ligand synthesis and redistribution in both pathways. The goal of upcoming empirical studies should be to learn more about the epigenetic regulation of the Hh and Wnt pathways. High-throughput technologies can be utilized to determine which epigenetic systems are overactive in different cancer types and provide biomarkers. These could help with the selection of epigenetic treatments for specific patient groups, as well as enhance outcomes and reducing toxicity.

Author contributions

SEN, **FN**, and **SV** wrote and compiled this article. **AAS** wrote and edited the manuscript comprehensively. All authors confirmed the final version of the paper.

Conflict of interest

The authors declare that they have no conflicts of interest.

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