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# Long Non-coding RNA *H19* Involvement in Cancer

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## Abstract

The rapidly progressing area of long non-coding RNA (LncRNA) is a constantly expanding and widespread field in both biology and medicine. The fact that most of the human transcriptomes have relatively no coding capacity. The gene encodes a predominantly cytoplasmic spliced, ~2.3kb long capped, and polyadenylated RNA that produces an unknown protein product. It belongs to a vastly persistent and imprinted gene cluster. It is singularly expressed on the maternal allele and it plays a vital part during genomic imprinting that occurs throughout growth and development. During embryogenesis the expression of the H19 gene is strongly induced and it is down-regulated after birth, the only exceptions being adult skeletal muscles and the heart. This gene is located on chromosome 7 in mice, and on chromosome 11p15 in humans. The first imprinted non-coding RNA (ncRNA) transcript that was recognized is H19. In the recent years, the expressions of this gene in several human cancers have been under investigation. Furthermore, the effect of H19 RNA on tumor development is also being researched. The H19 gene is a tumor suppressor that contributes towards cancer development and progression and accelerates cancer cell proliferation, metastasis, invasion, and apoptosis. Furthermore, the H19 gene can also act as an oncogene. Since the H19 gene plays a crucial role in several human cancers, this paper provides a comprehensive overview of our current knowledge on the H19 gene and provides further support towards the principal function of H19 RNA and the role it plays in tumorigenesis and the biological characteristics of the cancer pathways. The research results indicate that H19 is a prospective unique biomarker for the diagnosis, therapeutic targeting, and prognosis of cancers.

**Keywords:** LncRNA; H19; Cancer; Tumorigenic mechanism; Methylation; Pathway; Diagnosis.

## Introduction

According to the DNA tilling arrays and deep sequencing technologies human genome contains approximately 20,000 protein-coding genes that only account for about 1% of the total human genome. Over 90% of transcripts, including microRNA and long non-coding RNA (LncRNA), are non-coding RNA [1, 2]. LncRNA are over 200nt in length and are considered to be the "noise" for genomic transcription as its quantity, type, and function is ambiguous. Due to the lack of effective research methods, we shall refer to this transcribed and intergenic sequence as "dark matter" [3]. LncRNA are transcribed by RNA polymerase II (RNAPOII) and are often spliced and polyadenylated without an open reading frame and enriched in the nucleus or cytoplasm. They do not show evidence of protein-coding potential, but they can multi-dimensionally regulating gene expression similar to RNA [4]. LncRNA participates in X chromosome silencing, genomic imprinting and a variety of important regulatory process, such as chromatin modification, transcription activation, transcriptional interference, epigenetic regulation, etc. It has an important biological function in the occurrence and development of diseases. In addition, it also affects epigenetic regulation and other tumor cell growth, apoptosis, invasion, and metastasis.

Gene imprinting is a phenomenon that was discovered recently. Gene imprinting is independent of the Mendelian inheritance and it means that some genotypes are only inherited from the paternal or maternal chromosomes. When one parent-of-origin-specific allele is expressed, the other one will not be expressed or will only be expressed weakly [5, 6]. Generally, imprinted genes contain 3-12 genes that exhibit a clustered distribution and form one or a few Imprinting Control Regions (ICRs) such as Differentially Methylated Regions (DMRs) that are about 20-3700kb [7]. Studies show that a majority of imprinted genes exist in long non-coding RNA transcription with the long non-coding RNA achieving gene imprinting mainly through the Cis-transcriptional interference [8]. Furthermore, gene imprinting and its related long non-coding RNA abnormalities are associated with the occurrence of multiple tumors, while genomic imprinting caused by LncRNA plays a significant role in disease development and treatment.

*H19* is the first detected human LncRNA, and it is also one of the few imprinted genes that have been discovered up-to-date. Its paternal imprinting and maternal expression categorizes it into the development imprinted genes category. *H19* is a single copy gene, which is locates at the distal end of the $7^{th}$ chromosome in rats and on the 11p15.5 chromosome in humans. Human *H19* gene, which is 3kb long, contains 5 exons and 4 introns. Rsa polymorphism enzyme sites are on the fifth exon [9]. *H19* mainly exists in the cytoplasm and functions by regulating RNA or ribose [10]. *H19* mRNA contains 35 small open reading frames, among them, ORF6 is considered the largest as it starts at the first exon and ends at the second exon. Theoretically, it should be able to encode a protein with 256 amino acids. However, no related protein products have been found in the human body or cell lines. Moreover, no associations between *H19* and gene translation system have been detected. Considering these factors most researchers regard *H19* as the only discovered imprinted gene that acts as mRNA but does not express proteins [11]. It is widely expressed during the human and rat embryonic period, mainly in the endoderm and the ectoderm tissues. It partakes in the normal development of embryo and fetal behavior cultivation. After birth, the expression of *H19* remains only in the cardiac and skeletal muscles [12]. *IGF2* gene (Insulin-like Growth Factor 2) is an expression of the paternal and maternal allele imprinting. It is about 8.8kb and located on the 11p15.5 human chromosome with 9 exons, 8 introns, and p1-4 promoter [13]. Insulin-like growth factor produced by *IGF2* gene coding is not only a crucial embryonic cell growth promotion factor, but also a mitosis promotion peptide. It has a physiological effect on embryonic development but it can also stimulate proliferation of tumor cells. *H19* and *IGF2* belong to a common imprinted gene group that is highly conservative in evolution. *H19* is maternally imprinted, while *IGF2* is paternally imprinted. They have a 90 kb gap between them and they are both regulated by the DMR or ICR which is 4 kb upstream of *H19* [14].

## Regulatory Mechanism of Gene Imprinting

Most of the imprinted genes are regulated by a promoter, a boundary element, and a non-coding RNA. Approximately 10kb in the area upstream of *H19* there are several known regulatory elements some of which have important functions and are closely linked with the development of tumors. ICR, and DMR are both 4-2kb upstream of the *H19* transcription initiation region [15, 16]. *H19* contains methylated paternal and non-methylated maternal alleles.

## Promoter

The promoter of an imprinted gene is often rich in GC and is highly methylated in one of them. Therefore, the transcription factors cannot combine with the promoter to undergo transcription. The Methyl CpG binding protein (MeCP) can form complexes, such as Histone Deacetylase (HDAC) and histone acetylation that results in the histone H4 acetylation modification, which leads to chromosome structural contraction, and inhibition of gene transcription.

## Boundary Element

The methylation of DMR gene in most imprinted genes has a cis - repression effect which can lead to inactivation of the gene itself. However, there are a few exceptions where the methylation of the imprinted gene DMR will induce the activation of the gene. The role of DMR is dependent on the interaction between the isolated protein and the boundary element. For example, the CTCF factor is the boundary element CCCTC sequence specific binding factor. In the interactive imprint *IGF2* / *H19*, the center IC is located between *IGF2* and *H19* imprinting, which is about 2kb of the fragment. On the paternal allele, IC is methylated and the CTCF factor cannot combine with *H19*. This enhances the function of the *IGF2* gene, and *IGF2* starts transcription while the *H19* gene is suppressed. In the maternal allele IC is not methylated and the CTCF factor binds with *H19* and prevents the enhancement and interaction between the promoter and *IGF2*. This enhances the effect on the *H19* promoter, and the *H19* gene is transcribed while the *IGF2* gene transcription is inhibited.

## Non-encoding RNA Regulation

GTL2 and DLK1, the imprinting centers of human 14q32, regulate the expression of about 40 miRNA genes downstream of 200kb. All miRNA of this region are expressed by the maternal allele. DLK1 and GTL2 imprinted regions may be controlled by miRNA regulation [17].

## *H19* and Tumor

The loss of gene imprinting leads to the simultaneous expression of two alleles. Mutations causing the inactivation of active alleles are the cause of many diseases. Thus far it has been discovered that many kinds of tumors are related to abnormal gene imprinting. Regulation of gene imprinting plays an important role in the development and treatment of diseases. The following are the main contents of *H19* and tumor.

## Mechanisms of Tumorigenesis

It has been found that covalent modification of DNA methylation can lead to suppressor gene inactivation which leads to tumor occurrence. The range of gene mutations is extensive. Therefore, the research about the relationship between DNA methylation and tumors has become a hot spot in molecular biology [18].

DNA methylation is one of the initially discovered gene epigenetic modification. It may be present in all higher organisms. It is transferred from DNA methylation enzyme catalysis to s-adenosyl methionine as a methyl donor. The reaction with cytosine translates it into 5-methyl cytosine [19]. Methylation modification of DNA mainly occurs in the CpG islands that are rich in DNA fragments of CpG 300 ~ 3000bp. 40% of the base for the promoter region is within the CpG island [20]. Presently, DNA methylation is the main reason for the loss of gene imprinting, which occurs in the early stages of embryonic development in DNA methylation disorders, resulting in the loss of imprinting [21]. Methylation only occurs on the paternal alleles in the CpG islands upstream of the *H19* gene. In Wilms' tumor patients with Bi-allelic methylation resulting in the loss of imprinting, the *H19* gene is not expressed and the *IGF2* gene upstream of *H19* is over-expressed [22]. Thus, stimulating the formation of tumors. Studies have reported that through comparison of the DMR methylation degree of *H19* gene between HCC tissues and normal liver tissues, it was confirmed that the abnormal expression of *H19* could be caused by the methylation of the gene [23].

The factors affecting DNA methylation are DNA methyltransferase, histone methylation, and RNA1 interference. In mammalian cells, methylation of OpG MeCP2 binding proteins can not only promote histone acetylation, but also gene silence suppression while it is still a DNA and histone methylation bridge. MeCP2 can bind to the *H19* gene promoter during methylation of DNA and affect the histone H3 methyl transferase activity which prompts the histone lysine methylation of H3. The latter together with DNA methylation glycosylation plays a role of *H19* gene expression inhibition [24]. *H19/IGF2* gene abnormalities are associated with several types of cancer. Imprinting control regions (ICR) between *H19* and *IGF2* genes are present on the maternal chromosomes. ICR is non-methylated and as it acts as an insulator and a CTCF transcription binding factor (CCCTC-binding factor), it is able to block downstream enhancement of the binding promoter of the *IGF2* gene, leaving the promoter enhancer effect on the *H19* gene. This promotes the expression of *H19*. However, since the ICR on the paternal chromosome is methylated it cannot combine with the CTCF transcription factor. Therefore, it cannot play a role of an insulator. Downstream enhancement only promotes *IGF2* gene promoter binding. It does not promote *H19* gene promoter binding. The promotion of *IGF2* expression results in the inhibition of *H19* gene expression [25-27]. Meanwhile *H19* and *IGF2* expression is regulated by DMR. DMR is located 2 kb upstream of the *H19* gene. This area is rich in CpG. CpG methylation differentiates the regulation of *H19/IGF2* expression. Studies have found that DMR CpG methylation genes in prostate hyperplasia are present twice as much compared with prostate cancer. This suggests that DMR CpG methylation gene abnormalities may lead to abnormal expression of *H19*, thereby the occurrence of prostate cancer [28].

## Signaling Pathway

The exact role of intracellular *HI9* RNA is unclear. Although there are many substances and conditions known to activate transcription and the known *HI9* has a profound effect on the cell cycle activity and state, its action mechanism is not clear.

## Upstream Effect of Hormone Regulation

In a previous study Adriaenssens found that increased expression of *H19* is associated with steroid receptors. Further studies have also found that the main form of estrogen, l7-13-estradiol and corticosterone, can stimulate the transcription of *H19* in endometrial cells, but the effects of progesterone are inhibited. Tamoxifen is an estrogen receptor binding agent and is often used as a chemotherapeutic drug to treat breast cancer. 17-β-estradiol can stimulate *H19* transcription in MCF-7 cells alone, while increasing Tamoxifen inhibits the transcription of *H19*. This suggests that there is a putative hormone effect in the transcription of *H19* [29]. *H19* is highly expressed in malignant tumors, such as hepatocellular carcinomas, gallbladder cancer, and breast cancer. In colorectal cancer, the expression of *H19* is directly regulated by cmyc, suggesting that *H19* may be an effector molecule playing a regulatory role downstream of cmyc [30, 31]. Berteaux found that the expression of *H19* gene is regulated by the regulation of peptides and steroid hormones. These two hormones play opposite roles in the synthesis of *H19* RNA. This has been verified in LNCaP cells [32].

## Downstream Impact - Angiogenesis, Metabolism, Tissue invasion, and Migration

A comparative study on the variances in gene expression amid transfected HI9 cells and antisense transfected cells showed that there was an upregulation of the following genes: c-Src and uPar kinase, tyrosine kinase 2, tyrosine kinase 2 mitogen activated protein kinase, c-jun, JNK I, Janus kinase 1, intercellular adhesion molecule-1, TNFα, IL6, heparin-binding epidermal growth factor-like Growth Factor, and NF-kB [33]. In the cell and animal experiments of liver cancer and bladder cancer, Matouk et al. [36] found that FGF18 can promote cancer cell growth, increase hypoxia tolerance, and promote angiogenesis. They also identified the potential targets of *H19* transcription by angiopoietin and FGF18, which is also a downstream target of *H19* [31]. Based on the function of RNA gene and the gene expression of *H19*, it is considered that the *H19* gene plays a key role in tumor invasion, metastasis and angiogenesis.

## The Role of *H19* in Tumor

Some studies have demonstrated that the *H19* gene is highly expressed in some cancers, for example breast cancer [34], causing a carcinogenic effect. Other studies have shown that *H19* is expressed in a lesser degree in some cancers, such as liver cancer [35]. *H19* plays a dual role in carcinogenesis and tumor suppression in different tumors. This is mainly due to the dual functional characteristics of LncRNAs or it may be dependent on different backgrounds. However, further understanding of the precise function and biological function of *H19* is required [36].

## *H19* as A Proto-oncogene

The first exon of the *H19* gene can produce a highly conserved miRNA, miR-675H1, which is a precursor of *H19*. miR-675 acts on the RB mRNA 3' end untranslated region and inhibits the expression of the RB gene. This promotes tumor cells in colon cancer and primary colon cancer. The expressions of both of them were up-regulated at the same time. miR-675 affects RB mRNA at the 3' untranslated region and inhibits the expression of RB genemote promoting the growth of tumor cells [37]. miR-675 is highly expression in gastric cancer [38], breast cancer [39], lung cancer [40], and bladder cancer [41]. It has a role equivalent to that of an oncogene. In colon cancer [42] and glioblastoma [43], *H19* gene can be regulated by cmyc gene, and *H19* can be directly activated by oncogenic transcription factors and commonly influence the downstream gene expression. Dugimont reported that P53 gene can effectively inhibit the activity of the *H19* promoter [44]. In gastric cancer cells, *H19* can bind to P53 and inhibit the activity of P53. Thereby, reducing the level of Bax which is a downstream target gene of P53 and promote the proliferation and apoptosis of gastric cancer cells [45]. *H19* also regulates the transcription factor GLI1 activity, which is one of the key signal proteins in the formation of astrocytes. In human astrocytoma, the expression of GLI1 increases by 50 times, which displays the tumor promoting effect of *H19* [46].

## *H19* as A Tumor Suppressor Gene

In human colorectal cancer mouse model transplantation, the probability of occurrence of intestinal polyps in *H19* deficient mice was significantly higher than that of the wild type. Yoshimizu mainly used the teratoma model mice and the wild type *H19* knockout mice to detect the generated mice embryonic tumor weight, size, and tissue pathological differences. The results showed that the embryonic origin of tumors of the *H19* gene deficient mice is 1.6 times that of the wild type tumor size. This study also confirms that the *H19* gene can function as a tumor suppressor. In the liver cancer research model, the lack of the *H19* gene can promote tumor development or tumor enlargement, thus showing that *H19* plays a role in tumor suppression [47, 48].

Zhang et al. proved that the *H19* gene was expressed in HCC tissues and the low expression rate of *H19* in HCC tissues (T) / para carcinoma tissues (L) is often suggested as a poor prognosis. The mechanism is that *H19* can inhibit the metastasis of liver cancer by activating the miR -200 pathway.

## Invasion and Metastasis of Tumors and *H19*

Yesh and other studies have found that *H19* can regulate multiple genes. These genes are important in the development, invasion, and metastasis of tumor cells. The main causes of the tumor generation are the loss of imprinting, the loss of heterozygosity, single parent diploid, and abnormal changes in the control region of *H19* [33]. In gliomas, *H19* promotes the invasion of glioma cells by producing a direct effect on CDH13 of the miR-675 [49]. *H19* can promote the invasion, metastasis, and angiogenesis of bladder cancer cells by up-regulating the expression of p57 (kip2) [31]. A study found that *H19* was associated with hypoxic stress in tumor cells. Under hypoxic stress, the interaction of P53 with *H19* HIF1 (hypoxia-inducible) was 1 (factor-1alpha), and the cells were changed by adjusting the expression level of the cells. HIF-1 up-regulated *H19* expression and mediated cell adaptation to hypoxia, while P53 inhibited *H19* expression and enhanced hypoxia induced apoptosis, and the effect was determined by the state of P53 [50]. When there is a P53 mutation or deletion, *H19* expression increases to promote tumor progression (including angiogenesis, tumor metastasis, chemotherapy resistance, etc.). Abnormal protein acetylation of the *H19* promoter groups causes a down-regulation of *H19* and p300 / CBP associated factor expression. miR-200 family factors upstream of heterogeneous ribonucleoprotein U (heterogeneous nuclear ribonucleoprotein U, hnRNP U), and P300 / CBP-associated factors (P300 / CBP associated factor, PCAF) combined to reduce hnRNP U / PCAF / RNA Pol II protein complex histone acetylation weakening. This resulted in the reduction of miR-200 family acetylation of sub regional abnormal expression levels. Thus, weakening the negative regulation of miR-200 gene target zinc enhancer binding protein 1/2 (zinc finger E-box binding homeobox 1/2, ZEB1/2 inhibition) [35]. ZEB1/2 expression regulation will promote the epithelial mesenchymal transition (EMT), which can increase the ability of HCC cells to invade and migrate. In addition, *H19* by zeste homologous sequence 2 enhancer (enhancer of zeste homolog 2, EZH2) interacts to promote the EMT process [51]. Thereby, increasing the tumor cell migration and invasiveness. *H19* can promote tumor cell proliferation by DNA binding/differentiation 2 (binding/differentiation DNA 2, ID2), or directly induce the expression of cmyc and promote tumor development [52]. Additionally, *H19* was found to inhibit the invasion and migration of tumor cells by inhibiting Let7. Let7 is an effective tumor suppressor miRNA that regulates cell growth, movement, and the expression of cancer genes by transcriptional repression [53].

## *H19* and Tumor Diagnosis, Treatment and Recurrence Monitoring

LncRNAs have a close relationship with the occurrence of tumors. Therefore, the abnormal expression of LncRNAs in tumor cells may be used as a tumor marker for tumor diagnosis, especially in the early stage of some tumors. LncRNAs can regulate the expression of cancer associated proteins by gene silencing mediated at the transcription level. At this point, the diagnostic value of LncRNAs is even bigger, because the tumor tissue is small. It is difficult to distinguish by imaging studies. Thorough research on the mechanism of LncRNAs can be used to better understand its role in tumorigenesis, development, and provide a new target for the clinical treatment of tumors.

The function of *H19* RNA in cancer cells is not fully understood. Its presence in many types of tumor cells suggests that it can be used for initial diagnosis, and cancer recurrence monitoring. It can also be used as a malignant potential tumor marker in a clinical setting to aid in diagnosis [54, 55]. *H19* is not highly expressed in normal liver cells, but most of the time there is a significant increase in expression in primary liver cancer. The expression of LncRNA *H19* is significantly different and can improve the early diagnosis rate of liver cancer combined with the detection of AFP [56]. The loss of KCNQ1OT gene in blood cells and the high methylation of *H19* gene can be a diagnostic and prognostic judgment of Beckwith-Wiedemann syndrome [57]. Ariel discovered the relationship between the expression of *H19* in human bladder cancer and the early recurrence of bladder tumors. In this study 84% (47/56) of bladder cancer patients expressed the gene. It was found that the level of expression of *H19* decreased with the increase in tumor grade. The expression level of the tumor tissue was longer than that of the patients who expressed the gene. The *H19* gene can be used as a marker for the detection of early postoperative recurrence, and for the prevention or treatment of bladder cancer by regulating the gene sequence [58].

Activation of the *H19* promoter in cancer cells and silencing in normal cells is a recommended for the expression of cytotoxic genes that causes gene therapy using *H19* promoter to drive cancer cells. Experimental studies found that DTA (diphtheria toxin) expression vector *H19*-DTA-P4-DTA, a dual promoter, can be used to control *IGF2* and *H19* regulatory sequence to handle the dual promoter expression vector, so that there is a difference in cancer expression. This has the potential to reduce the tumor burden, to improve the quality of life for patients, and to prolong their life span by delivering drugs directly into the tumor cells [59]. Clinical trials on the combination of *H19* gene and DTA (DTA-*H19*) as immune treatment for bladder cancer, ovarian cancer, and pancreatic cancer have begun [60]. BC-819 or DTA-*H19* as a plasmid truly reflects the targeted therapy because all the plasmids enter the dividing cell.

But it can only be found by the *H19* transcription factor in tumor cells, which leads to the expression of DTA. Therefore, we can say that it only destroys tumor cells without affecting the function of normal cells. In a double center clinical trial where BC-819 was used as a superficial bladder cancer treatment regimen for phases I and IIa, more than 70% of the patients were not found to have any serious side effects towards the tumor treatment. This included the patients who have not yet taken the optimal dosage regime. BC-819 has been used as an experimental treatment for superficial bladder cancer, ovarian cancer, and metastatic liver cancer. A patient who had a complete bladder resection for bladder cancer did not have a recurrence of the tumor or any adverse effects after the treatment in 2004 [61]. An ovarian cancer patient was treated with BC-819. After the treatment the CA 125 level in blood was decreased by nearly 50%. For patients with metastatic liver cancer, BC-819 was injected directly into the tumor cells. Necrosis of part of the tumor cells was observed. Although the behavior of *H19* in most cancer types is well known, the part that *H19* RNA takes in the treatment of cancer cells is yet to be found. Nevertheless, recent studies have found that the behavior of p95 (NCA-90) in tumor cells was present in the presence of *H19* RNA [61, 62].

This theory can lead to the development of more personalized cancer treatment programs. For example, the overexpression of *H19* in tumor cells may indicate that there is a high tolerance towards drug toxicity. In patients who overexpress *H19* (and p95) radiotherapy or immunotherapy treatment can be given instead of chemotherapy. To sum up, the research on IGF-2 and *H19* in malignant tumor tissues may provide a new way for early diagnosis and prognosis of patients with malignant tumors.

## Conclusions and Perspectives

With the rising interest on the relationship between LncRNAs and tumors, more and more LncRNAs have been discovered. Their role and mechanism in tumor occurrence and development is also constantly updated. Based on the recognition of the role of *H19* LncRNA in tumor genesis, it can be used to design targeted agents for the role of tumor formation. This will provide an innovative method for molecular targeted therapy of tumors. Specific *H19* expression of tissues may be used as a tumor marker for tumor diagnosis. *H19* is closely related to the tumor stage, grade, and patient survival. In short, *H19* plays an important role in tumor genesis and development, and the research on the relationship between *H19* and tumors is expected to provide a new opportunity for tumor diagnosis and treatment. However, due to the diversity and complexity of the mechanism of *H19*, current understanding on the mechanism and function of *H19* is still rudimentary. DNA methylation plays an important role in the mechanism of *H19*. But there is no effective method to control and manipulate the methylation patterns. If we can find a method to effectively restore normal gene imprinting, it will no doubt give rise to a new way of thinking for prevention of cancer related tumors.

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