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REVIEW

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H19: An Oncogenic Long Non-coding RNA in Colorectal Cancer

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Colorectal cancer (CRC) has been recorded amongst the most common cancers in the world, with high morbidity and mortality rates, and relatively low survival rates. With risk factors such as chronic illness, age, and lifestyle associated with the development of CRC, the incidence of CRC is increasing each year. Thus, the discovery of novel biomarkers to improve the diagnosis and prognosis of CRC has become beneficial. Long non-coding RNAs (lncRNAs) have been emerging as potential players in several tumor types, one among them is the lncRNA H19. The paternally imprinted oncofetal gene is expressed in the embryo, downregulated at birth, and reappears in tumors. H19 aids in CRC cell growth, proliferation, invasion, and metastasis via various mechanisms of action, significantly through the lncRNA-microRNA (miRNA)-messenger RNA (mRNA)-competitive endogenous RNA (ceRNA) network, where H19 behaves as a miRNA sponge. The RNA transcript of H19 obtained from the first exon of the H19 gene, miRNA-675 also promotes CRC carcinogenesis. Overexpression of H19 in malignant tissues compared to adjacent non-malignant tissues marks H19 as an independent prognostic marker in CRC. Besides its prognostic value, H19 serves as a promising target for therapy in CRC treatment.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide, accounting for approximately 10% of all cancer cases, and is the second-leading cause of cancer-related death, with nearly 1-2 million new cases and 700,000-800,000 deaths per year [1-3]. By sex, CRC is the second most common cancer in women (9.2%) and the third in men (10%). Age past the fifth decade, chronic disease history, and lifestyle increase the risk of developing CRC, and men are more susceptible than women [2,4]. The morbidity and mortality rates of CRC remain high even as of 2023 [5]. Up to 95% of all CRC cases are adenocarcinomas of the colon and rectum. Despite the progress in early diagnosis and cancer therapy in the past decade, the overall survival rate of CRC remains unfavorable and low in the setting of metastasis [4,6].

CRC develops through a series of genetic modifica-

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Abbreviations: CRC, colorectal cancer; ncRNA, non-coding RNA; lncRNA, long non-coding RNA; miRNA, microRNA; ceRNA, competitive endogenous RNA; mRNA, messenger RNA; MRE, miRNA recognition element; CDS, coding sequence; 3'UTR, 3' untranslated region; circRNA, circular RNA; EMT, epithelial to mesenchymal transition; siRNA, small interfering RNA; RISC, RNA-induced silencing complex; SNP, single nucleotide polymorphism; ASO, antisense oligonucleotide; 5-Fu, 5-fluorouracil; VDR, vitamin D receptor.

Keywords: lncRNA, H19, CRC, ceRNA, miRNA sponge, miR-675, proliferation, EMT, prognosis, biomarker

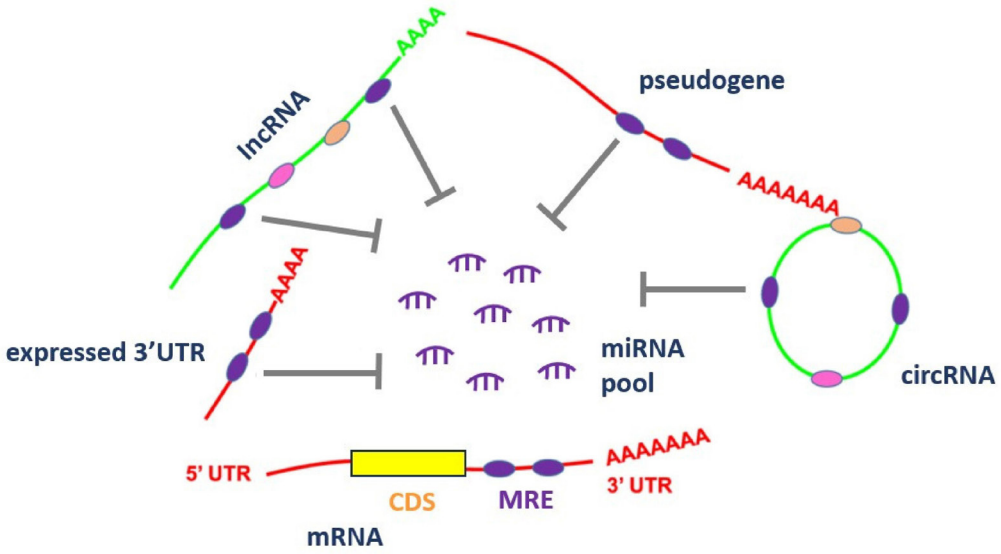

Figure 1. **ceRNA hypothesis**. mRNA contains MREs (represented by ovals), which are typically found within the 3'UTR. miRNA binds to identical MREs, and these MREs are present in various ncRNA species, including lncRNA, circRNAs, pseudogenes, and independently transcribed mRNA 3'UTRs. All of these RNA molecules compete for a limited pool of miRNA, thus exhibiting positive regulation of gene expression. lncRNA and circRNA may carry MREs for multiple miRNAs (represented by different colored ovals).

tions that transform normal colonic epithelium into an adenoma and ultimately adenocarcinoma. Environmental alterations and genetic mutations targeting oncogenes, tumor suppressor genes, DNA repair genes, or non-coding RNAs (ncRNAs) – long non-coding RNA (lncRNA) or microRNA (miRNA) are responsible for the occurrence and development of CRC [7,8]. Circulating levels of ncRNAs in plasma or serum has been an emerging field for non-invasive diagnostic applications for cancer [9,10]. Notably, stable expression of ncRNAs has exhibited their potential predictive value when used as biomarkers, thus gaining much interest recently [2,11-13].

ncRNAs, transcribed from the non-coding regions of the genome, lack an open reading frame and are not translated into proteins [14]. lncRNAs (>200 nucleotides in length) modulate gene expression at the epigenetic, transcriptional, and post-transcriptional levels in the nucleus and cytoplasm. It has been implicated in crucial biological functions involved in cancer susceptibility [15-18]. Under physiological conditions, lncRNAs regulate gene expression, epigenetic printing, and alternative splicing, or may act as proto-oncogenes or tumor suppressor genes. Their overexpression as proto-oncogenes leads to an upregulation of genes involved in tumor progression, whereas as tumor suppressor genes, they regulate the expression of *P53*-dependent genes [19-21]. A reduction in the expression of lncRNAs alters the expression of genes under their control, thereby making cells resistant to apoptosis and increasing their proliferation [20].

One of the central players in both embryogenesis and tumorigenesis is the oncofetal lncRNA H19. Found close to the telomeric region of chromosome 11p15.5, the 2.7 kb H19 gene is paternally imprinted and maternally expressed. It is reciprocally imprinted and regulated with its neighboring gene *IGF2* [22]. H19 is overexpressed in several solid tumors [22-29]. It acts as an oncogene to trigger the onset and progression of malignancies through its ability to augment the proliferation rate and the invasive and migratory potential of cancer cells *in vivo* [30,31].

H19 has key regulatory functions in CRC initiation and progression. It functions as a molecular competitive endogenous RNA (ceRNA) sponge for miRNAs involved in the manipulation of CRC malignant phenotypes. miRNAs regulate messenger RNA (mRNA) by binding to miRNA recognition element (MRE) motifs in the coding sequence (CDS) or 3' untranslated regions (3'UTRs) of target mRNAs, leading to their degradation or translation repression. Other ncRNAs such as lncRNA, circular RNA (circRNA), and pseudogenes and independently transcribed 3'UTR regions of mRNA also contain such motifs that can bind to miRNAs (Figure 1). Specifically, when H19 acts as a ceRNA sponge, the translation activity of miRNAs is sequestered, where H19 containing similar MRE motifs found in the 3'UTRs of mRNAs, interact with miRNAs, effectively sponging up or sequestering the miRNAs away from their target mRNAs. Consequently, H19 leads to increased stability and regulates

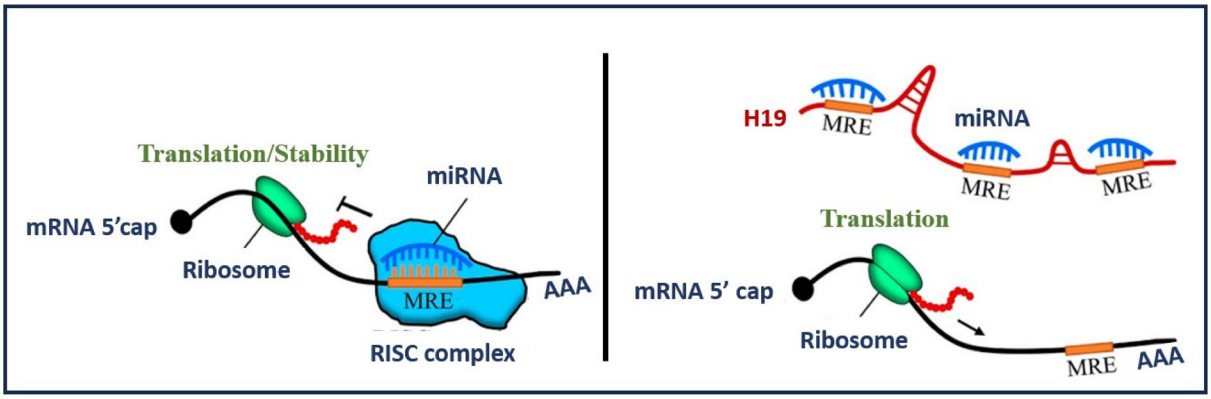

Figure 2. **H19 as a ceRNA sponge for target miRNAs**. miRNAs in CRC bind to the 3'UTR of target mRNAs associated with tumor progression and control their expression post-transcriptionally, which involves either inhibiting translation or reducing mRNA stability. H19 acts as a miRNA sponge through its MRE binding sites, thereby impeding the mRNAs' ability to regulate their target genes.

gene expression of target mRNAs (Figure 2) [32]. H19 regulates the expression of mRNAs involved in CRC cell proliferation, differentiation, and epithelial-to-mesenchymal transition (EMT) essential for CRC lesion metastasis to secondary sites [33,34]. Moreover, alterations in H19 remain constant among CRC patients, thus constituting a potential CRC biomarker [2,35]. H19 exhibits stable expression in these tumor cells, contributing to its favorable prognostic value and driving tumorigenesis through proliferation imbalance, translational deregulation, and metastasis. Although H19 may have been studied as a potential prognostic marker in CRC, research on this lncRNA's clinical significance remains low and needs further investigation. This review focuses on recent insights into the mechanisms of action of H19 leading to the development of CRC, anticipating poor prognosis and chemoresistance, and reflections on how H19 could be beneficial when used as a prognostic marker and therapeutic target.

FUNCTIONS OF H19 AND ITS SUPPRESSION MECHANISM

H19 has a highly conserved secondary structure and its function is structure-dependent [36]. H19 essentially has two major functions: (i) a modulator of miRNAs and proteins via their binding, and (ii) a reservoir for miR-675 that suppresses its targets [22,37]. In the nucleus, H19 controls chromatin modification proteins to regulate chromatin remodeling, epigenetic markers, and gene expression in cis or trans (Figure 3) or behaves as a splicing regulator, while H19 in the cytoplasm modulates translation and protein activity or mediates mRNA degradation [15]. The oncogenic properties of H19 are supported by its involvement in cellular proliferation and differentiation along with transitions between the epithelial and mesenchymal cellular phenotypes [22].

The association of H19 in tumorigenesis and invasion is partially owed to its regulation of highly conserved carcinogenic miR-675, a transcript of the H19 locus residing within the first exon of the H19 gene [22,38]. miR-675 is a short ncRNA whose primary miRNA precursor is H19 [39-42]. Exon 1 of the H19 gene contains a hairpin structure that serves as the blueprint for the two distinct forms of miR-675, miR-675-5p, and miR-675-3p. The miR-675 stem loop within the H19 gene is the most conserved region and these miRNAs may confer functionality on H19 [37]. However, the production of miR-675 from H19 represents a specific mechanism of gene regulation that works independently of H19's role as a sponge for other miRNAs. miR-675 also regulates gene expression by either binding to target mRNA and inhibiting translation or promoting mRNA degradation. Both H19 and miR-675 are upregulated in CRC [38,39]. H19-derived miR-675 regulates the development of CRC via the downregulation of tumor suppressor gene *RB1* and other transcripts in a cellular context-dependent manner; H19-facilitated miRNA decoy initiates chronic cellular proliferation and invasion, both of which are intimately involved in cancer [2,15,43]. While H19/miR-675 typically supports the growth of tumor cells, it exhibits cell-type specific functions, where it constrains cell growth through regulated processing of miR-675. Under cellular stress or oncogenic signals, the controlled excision of miR-675 has been observed to facilitate the inhibition of cell proliferation by the regulation of the growth-promoting *IGF1r* gene [37].

Suppression of H19 is modulated by tumor suppressor gene *P53*. Since H19 is a tumor promoter and P53 is a tumor suppressor, H19 and P53 are mutually count-

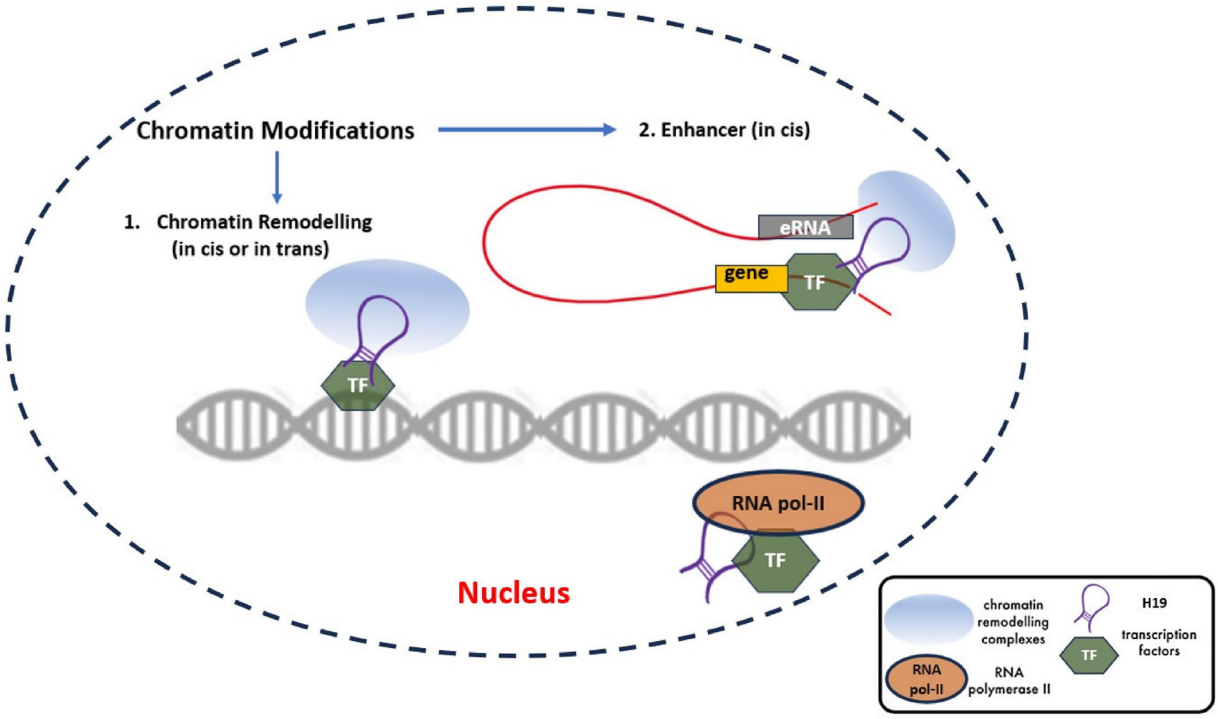

Figure 3. **Chromatin modifications by H19**. H19 within the cell nucleus engage in interactions with chromatin remodeling factors and transcription factors, thereby orchestrating the control of nearby and distal gene expressions.

er-regulated. Nuclear localization of P53 inhibits H19 by repressing its promoter activity and inducing DNA methylation of the upstream imprinting control region of the H19 gene, therefore resulting in epigenetic suppression of H19 expression *in vivo* [43-46]. The opposite mechanism has been reported in *P53*-impaired cancer cells, both *in vitro* and *in vivo,* where hypoxia as a stress stimulus triggers HIF1-α mediated H19 transcription, thus driving carcinogenesis through suppression of cyclin-dependent kinase inhibitor and other tumor suppressor genes, while upregulation of pro-oncogenic genes [43,47].

H19 suppression is also mediated by small interfering RNA (siRNA) *in vitro.* Single-stranded si-H19 with the RNA-induced silencing complex (RISC) binds complementarily with H19 forming a double-stranded RNA duplex. siRNA-targeted RISC complex induces degradation of H19, thus silencing the expression of H19 in tumor cells. Studies have demonstrated that siRNA-induced H19 suppression inhibits cell motility and invasion with decreased SNAI1 and increased E-cadherin expressions, thus indicating partial reversion of the EMT process [48,49].

ROLES OF H19 IN CRC

H19 upregulation in CRC tissues correlates with tumor differentiation and tumor node metastasis. H19 acts as a ceRNA for miRNAs to indirectly regulate its downstream target mRNA genes, recruit proteins or work through its derivative miR-675, and significantly potentiate tumorigenicity and metastasis of CRC, both *in vitro* and *in vivo* [50]. Following the H19-miRNA-mRNA regulatory network, the role of H19 in CRC carcinogenesis and prognosis is discussed below. Table 1 lists the different molecular pathways through which H19 is associated with the malignancy of CRC. Figure 4 summarizes the known molecular mechanisms by which H19 regulates colorectal tumor growth and metastasis. Table 2 gives a brief comparison of the roles of H19 in cellular proliferation and metastasis in different tumor types.

H19 Mediates CRC Cell Growth and Proliferation

Notable overexpression of H19 in tumor cells accelerates cell cycle progression through upregulation of cell cycle regulatory genes and inhibition of apoptosis. Reciprocally, H19 depletion induces growth arrest by inhibiting cell viability [51].

CRC cell development requires the interaction of H19 with the eukaryotic initiation factor, RNA-binding protein eIF4AIII [51,52]. eIF4AIII serves as a major RNA binding constituent with protein MLN51 within the core of the exon junction complex [53-55]. eIF4AIII-formed exon junction complex modulates protein translation and

Table 1. Mechanism of Action of H19 Associated with CRC Progression

Signaling network	H19 action on miRNA or protein	Molecular mediator	Effect	Reference
H19/eIF4A3/CDK4/ cyclin D1/E1	inhibitor for eIF4A3 protein	upregulation of cyclin D1, cyclin E1, CDK4	tumor cell growth, proliferation, and differentiation	[51]
H19/miR-200a/β-catenin	ceRNA for miR-200a	upregulation of β-catenin		[60]
H19/CDK8/β-catenin	promoter for CDK8	upregulation of β-catenin		[61]
H19/miR-200a/ZEB	ceRNA for miR-200a	upregulation of ZEB1, ZEB2 downregulation of E-cadherin	tumor cell invasion and metastasis through EMT	[34]
H19/miR-138/Vimentin	ceRNA for miR-138	upregulation of Vimentin downregulation of E-cadherin		[34]
H19/miR-29b-3p/PGRN/ Wnt signaling	ceRNA for miR-29b-3p	upregulation of PGRN, Vimentin, SNAI1 downregulation of E-cadherin		[82]
H19/Ras/RAF/MEK/ ERK	promoter for Ras/MAPK signaling	activation of Ras p-RAF, p-MEK, p-ERK		[72,73]
H19/miR-22-3p/HDAC2/ MMP14	ceRNA for miR-22-3p	loss of HDAC2 upregulation of MMP14		[6]
H19/HNRNPA2B1/*RAF*-ERK signaling	promoter for HNRNPA2B1 protein	upregulation of HNRNPA2B1		[85]
H19/miR-194-5p/FoxM1	ceRNA for miR-194-5p promoter for FoxM1	upregulation of Vimentin, N-cadherin downregulation of E-cadherin	increase tumor cell invasion and metastasis, function as a prognostic biomarker	[96,97]
H19/miR-675/*RB1*	repressor for *RB1* gene	downregulation of *RB1,* E2F phosphorylation of cyclin D1, CDK4	increase tumor cell proliferation, function as a therapeutic target	[98]
H19/miR-141/β-catenin	ceRNA for miR-141	upregulation of β-catenin		[128]
H19/miR-194-5p/SIRT1	ceRNA for miR-194-5p	repressed activity of SIRT1	5-Fu chemoresistance in tumor cell	[88]
H19/miR-675-5p/VDR	precursor for miRNA-675-5p	downregulation of VDR	1,25(OH)2D3 resistance in tumor cell	[118]

post-translational changes by triggering nonsense-mediated mRNA decay [56]. H19 binds to eIF4AIII, obstructing the recruitment of eIF4AIII in the mRNA regions of CDK4, cyclin D1, and cyclin E1 and inhibiting the mRNA decay of these cell cycle regulatory genes. As a result, these cyclin-dependent kinases mediate CRC cell division and regulate proliferation through the H19/eIF4AIII/CDK4/cyclin D1/E1 pathway [51].

CRC tissues exhibit elevated levels of H19 expression, compared to normal colon tissues. Additionally, this overexpression facilitates CRC cell proliferation via overactive β-catenin expression, whereas proliferation is significantly suppressed by H19 knockdown due to decreased expression of β-catenin [57]. H19 promotes cell proliferation through the comprehensive Wnt/β-catenin signaling by functioning as a miR-200a decoy in the miR-200a/β-catenin pathway [58,59]. miR-200a is a downstream target of H19 that inhibits cell proliferation through β-catenin expression repression in CRC. β-catenin is endogenously targeted by miR-200a resulting in repressed expression and activity of β-catenin in CRC cells and lower proliferation, but competitive binding of H19 to miR-200a antagonizes the function of miR-200a, causing de-repression of β-catenin expression, thereby promoting cell proliferation and EMT progression [60].

Further, β-catenin expression-induced growth and

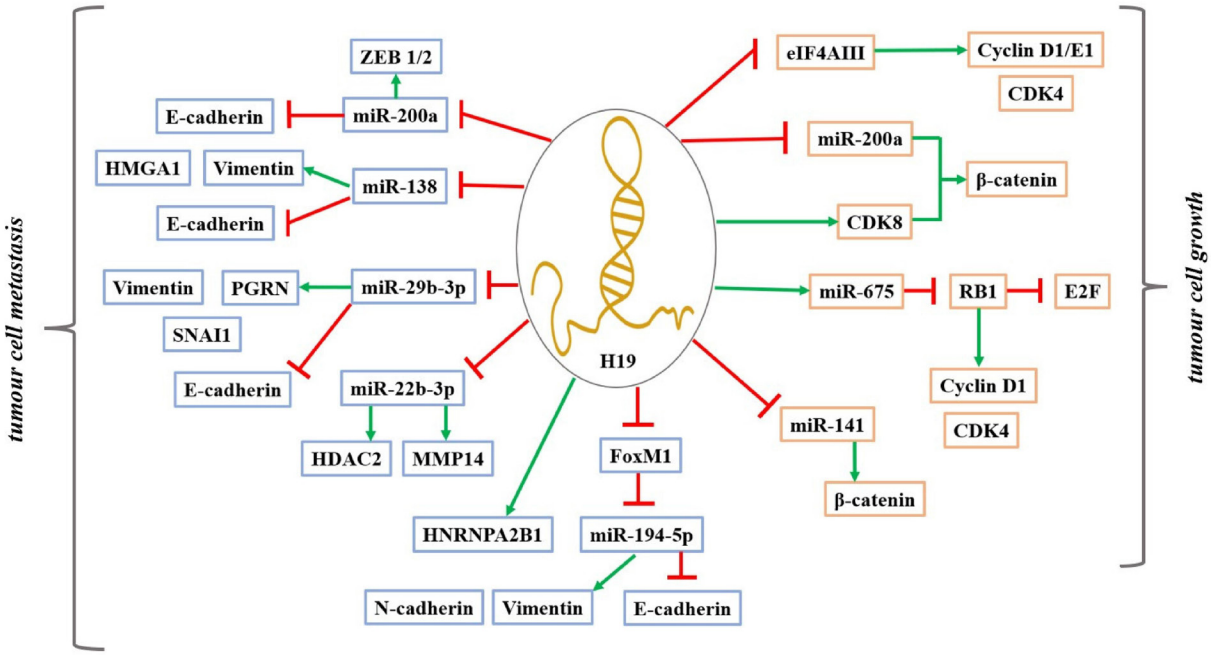

Figure 4. **Schematic diagram of the molecular mechanisms of H19 in the regulation of CRC growth and metastasis**. H19 typically acts as a ceRNA sponge for miRNAs, thereby regulating downstream expressions in CRC progression. H19 also acts as a promoter or repressor lncRNA that modulates the functions of specific proteins intimately involved in CRC.

proliferation of tumor cells is mediated by CDK8, an oncogenic driver of H19 in CRC. CDK8 gene is amplified in CRC. Through the CDK8/β-catenin pathway, H19 regulates the transcription of CDK8, which consequently activates the effect of β-catenin in CRC tissues. β-catenin expression drives the growth and proliferation of CRC cells and paves the pathway for EMT progression in CRC [61].

H19 Mediates Tumor Invasion and Metastasis in CRC

CRC cells metastasize to distant sites promoting cancer progression by acquiring mesenchymal phenotype through EMT [62]. In EMT, epithelial cells lose cell-to-cell adhesion and gain motile and invasive characteristics [34]. This transformation is influenced by transcription factors like TWIST, ZEB, and SNAI1, which alter the cells' features, allowing them to migrate through tissues, enter the bloodstream, and metastasize to secondary sites [63-67]. EMT is facilitated by the loss of calcium-regulated adhesion molecule E-cadherin in epithelial tumor cells, and concomitant increased expression of mesenchymal marker Vimentin and actin stress fibers in colon epithelium [68]. Stable expression of H19 serves as a positive regulator of EMT not only in CRC but also in other tumors of epithelial origin [69-71]. In CRC cells, it accelerates *in vitro* and *in vivo* invasion by functioning as a ceRNA sponge through the de-repression of mesenchymal marker genes modulated by H19-targeting miRNAs, and activation of prominent signaling pathways such as the Wnt signaling and Ras-RAF-MEK-ERK pathway [72,73].

H19 by de-repression of the function of ZEB and Vimentin facilitates EMT in CRC cells [74-76]. EMT-related transcription factors ZEB1 and ZEB2 bind to E-boxes within the promoter region of E-cadherin through their zinc-finger domains to reduce the expression of E-cadherin and other epithelial junction genes to facilitate EMT [63]. Ectopic expression of miR-200a and miR-138 in these cancer cells significantly diminish the mRNA and protein levels of ZEB1/2 and Vimentin, respectively, thereby attenuating EMT [74,75,77]. H19 in these cells, however, competitively sponges miR-200a and miR-138, antagonizing their inhibition on the core mesenchymal marker genes ZEB and Vimentin and subsequently proceeding differentiation from epithelial to mesenchymal cells [34].

Moreover, miR-138 acts as a regulator in inhibiting cell invasion and increasing the sensitivity of cancer cells to chemotherapy. In CRC, miR-138 prevents cell invasion and migration by restraining EMT through the downregulation of Vimentin as mentioned above, or even by repressing the function of the HMGA1 protein. HMGA1 is crucial in many biological processes such as

Table 2. Association of H19 in Different Tumor Types

Cancer type	Signaling network	Effect	Reference
Bladder cancer	H19/miR-675/p53	H19 promotes miR-675 expression to silence p53 expression in preventing cell death	[129]
	H19/miR-29b-3p/EMT	H19 acts as a sponge and reduces miR-29b-3p expression to induce EMT in increasing progression and invasion of cancer cells	[29]
Gastric cancer	H19/miR-519d-3p/LDHA	miR-519d-3p down-regulation by H19 to increase LDHA expression increased proliferation glycolysis induction	[130]
	H19/Wnt/β-catenin	induction of Wnt signaling by H19 to promote EMT induction in cancer cells	[131]
Breast cancer	H19/miR-130a-3p/SATB1	miR-130a-3p sponge by H19 in increasing SATB1 expression apoptosis inhibition facilitating tumor growth	[132]
	H19/p53/TNFAIP8	H19 reduces p53 expression to upregulate TNFAIP8 suppresses TNF-mediated apoptosis by inhibiting caspase-8 activity promotes EMT and cancer metastasis	[133]
Hepatocellular cancer	H19/miR-15b/CDC42/PAK1	H19 sponges miR-15b to induce CDC42/PAK1 axis hepatocellular carcinoma proliferation	[134]
Nasopharyngeal cancer	H19/miR-675-5p/SFN	H19 promotes miR-675-5p expression to downregulate SFN in decreasing the progression of cancer cells	[135]

transcription, differentiation, and neoplastic transformation and is found upregulated in many neoplasms [78-80].

The Wnt signaling is a key cascade tightly associated with cancer [81]. H19/miR-29b-3p/PGRN axis is another pathway through which H19 induces EMT in CRC cells by acting on the Wnt signaling. The expressions of H19 and its downstream target miR-29b-3p within CRC cells are negatively correlated [82]. miR-29b-3p directly represses PGRN, a protein known to promote EMT in multiple cancer cells [83]. This alters the downstream Wnt signaling, notably upregulating E-cadherin expression and downregulating c-Myc, Vimentin, and SNAI1 expressions constraining EMT, but owing to the high expression of H19 in CRC reverses this regulation of EMT markers by targeting miR-29b-3p to inhibit its expression and facilitate EMT and metastasis [82].

The Ras/MAPK signaling pathway is another important signaling in human cancer. In this pathway, the activation of Ras initiates the activation of RAF. Activated RAF subsequently phosphorylates and activates MEK, which phosphorylates and activates the MAPK/ERK [84]. H19 promotes the migration and invasion of CRC cells by stimulating the Ras/MAPK signaling. Overexpression of H19 in these tumor cells elevates the expression of active Ras, a guanosine triphosphate-binding protein that is one of the most common upstream molecules in signaling pathways. Consequently, activated Ras protein phosphorylates and increases the expressions of p-RAF, p-MEK, and p-ERK, thus leading to potential stimulation of metastasis in CRC by H19 through the H19/Ras/RAF/MEK/ERK signaling pathway [72,73].

HDAC2 and HNRNPA2B1 are epigenetic regulators that are associated with H19 to regulate EMT in CRC. Expressions of HDAC2 and HNRNPA2B1 have respective effects on the expression of H19 in CRC tissues. Reduced HDAC2 expression is associated with CRC metastasis and poor prognosis [6]. Depletion of HDAC2 inhibits deacetylation of histone H3K27 in the promoter region of H19 and induces EMT by upregulating H19. H19 functions as a miR-22-3p sponge to increase the expression of the MMP14 enzyme to promote migration. However, CRC metastasis is suppressed by H19 knockdown, despite the loss of HDAC2 [6]. On the contrary, activation of HNRNPA2B1 within CRC cells amplifies H19-induced migration [85]. H19 binds to HNRNPA2B1, and the interaction between HNRNPA2B1 and *RAF-1* mRNA further facilitates metastasis via stable upregulation of the expression of *RAF-1*. Increased levels of RAF-1 protein phosphorylate ERK, result in activation of the *RAF*-ERK signaling in H19/HNRNPA2B1-mediated EMT. Phosphorylated ERK also stimulates the production of SNAI1 protein, which further facilitates the EMT process (Figure

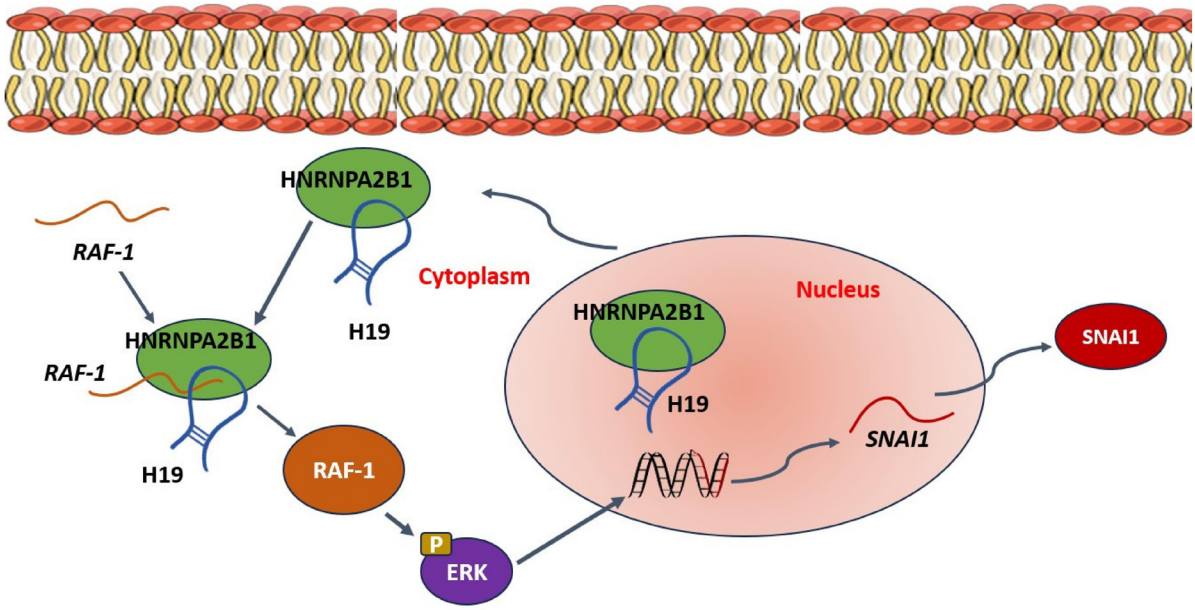

Figure 5. **Mechanism of H19 interaction with HNRNPA2B1 involved in CRC metastasis**. H19 interacts with HNRNPA2B1, resulting in the translocation of HNRNPA2B1 from the nucleus to the cytoplasm. This facilitates the binding between HNRNPA2B1 and RAF-1 mRNA, stabilizing the mRNA and consequently leading to the upregulation of RAF-1 that activates the ERK signaling pathway. This activation, in turn, promotes the transcription of SNAI1, inducing the process of EMT.

5) [85]. Therefore, the downregulation of metastasis suppressor HDAC2 and upregulation of metastasis promoter HNRNPA2B1 in CRC tissues bring about H19-induced migration and invasion through EMT.

Clinical Applications of H19

H19 is believed to be the most significant lncRNA associated with CRC prognosis on account of its relevance with CRC survival and due to its abundance and stability in primary tumor and metastatic tissues that correlate to the disease's poor prognosis linking to tumor grade, lymph node metastasis, and distant metastasis identified by TNM staging [86,87]. Quantitative analysis has shown that H19/miR-675 is remarkably increased in recurrent CRC patient samples [88]. Elevated H19 expression, along with higher miR-675 levels and the presence of lymph node metastases, were identified as independent prognostic factors associated with unfavorable disease-free survival [89,90]. Studies have revealed that individuals exhibiting high H19 levels experience shorter overall survival and lower recurrence-free survival rates over 4 years [51]. Examining the presence of serum exosomal H19 has recently emerged as an innovative method for detecting CRC through liquid biopsy biomarkers. Nonetheless, it has been observed that the expression of H19/miR-675 stimulates skeletal muscle differentiation and regeneration [91]. This effect could introduce background interference when attempting to detect H19 in CRC. Therefore, additional research is imperative to develop a precise method for detecting exosomal serum H19 in CRC.

Another potential prognostic biomarker for predicting CRC susceptibility is the presence of H19 polymorphism at a single nucleotide. Single nucleotide polymorphism (SNP) may serve as prognostic as well as predictive markers in CRC therapy, because of their involvement in genetic pathways associated with metabolism, cellular transport, and the modes of action of chemotherapeutic agents, potentially impacting treatment response [92,93]. SNP rs2839698 in H19 is associated with the increased occurrence and development of CRC by alteration of specific structural motifs of H19. The SNP rs2839698 is located in the 3'UTR of the exon of the H19 gene which exerts effects on H19 expression and causes functional changes in its promoter activity [94]. This leads to alterations in H19-targeted miRNAs, therefore modulating the risk and development of CRC [95].

The presence of low levels of miR-194-5p, coupled with high levels of H19 expression has emerged as an independent prognostic marker in CRC cells due to its role in promoting metastasis through the H19/miR-194-5p/FoxM1 axis. miR-194-5p has the effect of elevating E-cadherin expression and concurrently reducing the expressions of Vimentin and N-cadherin. However, H19 targets miR-194-5p in CRC cells as a sponge for this

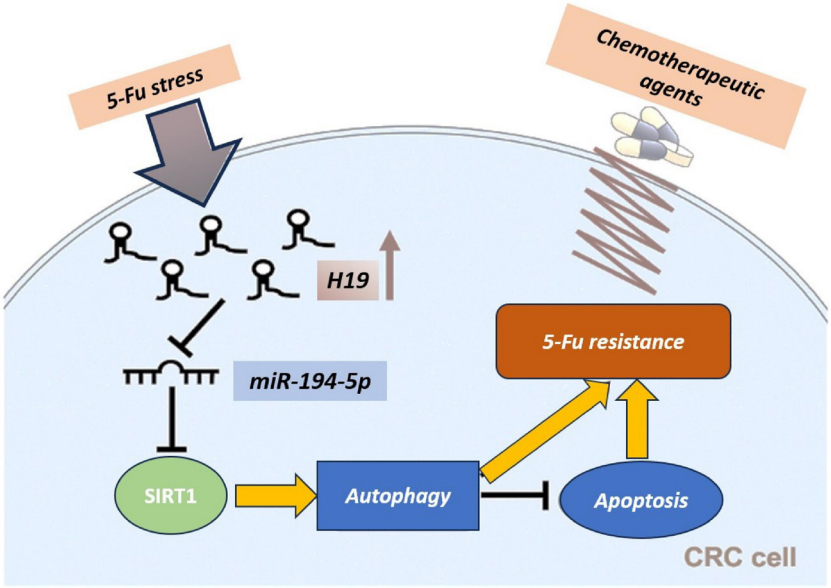

Figure 6. **The functional model underlying the mechanism of H19 in 5-Fu chemoresistance**. In response to 5-Fu stress due to chemotherapeutic agents, overexpression of H19 in CRC cell sponges miR-194-5p, which, in turn, inhibits SIRT1-mediated apoptosis and leads to tumor growth by autophagy.

miRNA, thereby inhibiting its expression. Additionally, FoxM1 expression in these cells positively correlates with H19 expression. Yet, its negative association with the expression of miR-194-5p ultimately reverses the inhibitory role of miR-194-5p in the invasion, migration, and EMT of CRC cells, characterized by a notable rise in Vimentin and N-cadherin levels, coupled with a reduction in E-cadherin expression [96,97].

H19-derived miR-675 serves as a target for CRC therapy via the molecular H19/miR-675/*RB1* pathway in CRC [98]. As aforementioned, oncofetal H19 is the primary miRNA template for miR-675 and both promote CRC tumorigenesis [40]. Their oncogenic function targets tumor suppressor *RB1* and E2F and affects the *RB1*-E2F signaling that controls the G_1 to S phase transition. Transcription factor E2F is a cell cycle regulator of *RB1*. In CRC cells, overexpressed miR-675 attenuates the *RB1* gene, which is a downstream target gene of miR-675 [87]. The effect of miR-675 on *RB1*-E2F controls the transcriptional regulation of the two immediate upstream regulators of *RB1* phosphorylation, CDK4, and cyclin D1 [99]. Therefore, the inactivation of *RB1* due to miR-675 hinders the interaction between *RB1* and E2F leading to aggressive tumor proliferation by phosphorylation of cyclin D1 and CDK4 and cell cycle dysregulation. Phosphorylated cell cycle regulators also contribute to the malignant transformation of CRC to a certain degree [99-102]. Thus, H19/miR-675 could be used as a prognosis marker as well as a potential therapeutic target in CRC due to its role in regulating the development of this cancer through the downregulation of *RB1*.

In addition to CRC, H19 has emerged as a potential novel target for therapeutic strategies in various cancer types. One promising approach is antisense oligonucleotide (ASO) therapy, which aims to reduce H19 expression in tumor cells *in vivo*. Recent evidence has shown that ASOs can disrupt H19 expression through premature transcriptional termination besides their effectiveness in promoting RNase H-mediated degradation of mature RNA, particularly in lymphoma. These findings underscore the potential of ASO therapy as a valuable tool for modulating H19 expression in cancer treatment strategies [103,104].

Chemotherapy failure in CRC patients is the major cause of its recurrence and poor prognosis. Cytotoxic chemotherapy drug 5-fluorouracil (5-Fu) is extensively used as a classic drug for adjuvant and palliative chemotherapy in CRC treatment. 5-Fu blocks normal DNA synthesis and disrupts RNA processing by targeting thymidylate synthase, producing anticancer effects as a result [105]. lncRNAs have exhibited an important role in tumor drug resistance via autophagy of tumor cells [106,107]. In tumor cells, autophagy suppresses tumorigenesis by inhibiting their survival and inducing cell death, but it also facilitates tumor growth by stimulating their proliferation [108-110]. Under metabolic and therapeutic stress, autophagy supports the survival of tumor cells by sequestering organelles and proteins in autophagic vesicles and delivering cytoplasmic cargo to lysosomes for degradation [101,109,111,112]. In response to 5-Fu treatment, protective autophagy reduces apoptotic cell death of CRC cells, and the inhibition of autophagy aug-

ments 5-Fu chemotherapy [113].

H19 confers 5-Fu chemoresistance in CRC cells via regulation of the autophagy regulator SIRT1 and sponging of miR-194-5p when tumor cells are exposed to 5-Fu-induced stress (Figure 6). It accomplishes this by reducing cell death and notably increasing the levels of autophagy marker LC3-II. The overexpression of H19 upregulates the conversion of LC3-I to LC3-II leading to a marked increase in LC3 aggregation and autophagosome formation in CRC cells while downregulating the expression of the autophagy receptor protein p62 [88]. H19 harbors a recognition sequence of miR-194-5p, and SIRT1 is a potential target gene of miR-194-5p at its 3'UTR [114,115]. SIRT1 is a NAD^+-dependent histone deacetylase [116]. H19 forms the miRNA ribonucleoprotein complex through competitive binding with miR-194-5p, counteracting the repressive activity of miR-194-5p on SIRT1 expression, thereby exerting 5-Fu chemoresistance promoting functions in CRC, through H19/miR-194-5p/SIRT1-dependent autophagy pathway. Importantly, ectopic expression of miR-194-5p significantly reverses the H19-induced resistance to 5-Fu and neutralizes the apoptosis-inhibiting effects exerted by H19 in CRC cells. Therefore, in CRC cells resistant to 5-Fu, H19 may be a potential target for therapy and may function as a predictive marker of chemotherapeutic response to 5-Fu [88].

High serum levels of vitamin D have been shown to lower the incidence of CRC. The active form of vitamin D, 1,25(OH)2D3 has pro-apoptotic and anti-inflammatory effects, in addition to inhibiting Wnt/β-catenin signaling, to decrease the growth and differentiation of colon epithelial cells [117]. Vitamin D receptor (VDR) signaling plays a pivotal role in attenuating the initiation and proliferation of CRC [118]. However, the overexpression of H19/miR-675 underlies the development of resistance to the treatment with 1,25(OH)2D3 in the advanced stage of CRC. While VDR signaling effectively suppresses H19 expression by controlling the C-Myc/Mad-1 network, the overexpression of H19/miR-675, on the other hand, hinders the expression of VDR through miR-675-5p. miR-675-5p binds to the 3'UTR of VDR mRNA and significantly decreases the expression of VDR in these tumor cells, thereby inducing resistance to 1,25(OH)2D3 treatment [118].

Furthermore, crosstalk between carcinoma-associated fibroblasts and cancer cells in the tumor microenvironment through exosomes shapes the tumor environment, promotes tumor development and metastasis, and confers chemoresistance [119,120]. lncRNA-loaded exosomes serve as mediators to regulate the tumor microenvironment, stemness, and chemoresistance of cancer cells [121-125]. In CRC, H19 expressed by carcinoma-associated fibroblasts of the colorectal tumor stroma contributes to the progression of the tumor and chemoresistance. H19 is transferred from carcinoma-associated fibroblasts to cancer cells through exosomes, and exosome-enriched H19 promotes the stemness of CRC stem cells and chemoresistance of CRC cells, both *in vitro* and *in vivo*. Overexpression of H19 in CRC cells significantly enhances the expression of pluripotency transcription factors and intracellular CRC stem cell marker ALDH1 enzyme, thereby contributing to CRC stem cells' self-renewal and pluripotent capacity. In addition, H19 activates the β-catenin pathway by acting as a miRNA sponge for miR-141 in CRC cells, sequestering the inhibitory effect of miR-141 on stemness and inducing proliferation by β-catenin [126-128]. Exosomal H19 from CRC stroma also decreases the chemosensitivity of H19-overexpressed CRC cells to the chemotherapeutic drug oxaliplatin through apoptosis, thereby allowing CRC cells to confer resistance to oxaliplatin-based therapy [128]. Therefore, targeting H19 in chemoresistant CRC cells could be a promising pathway for therapy.

CONCLUSION AND FUTURE PERSPECTIVES

This review focused on the main stations of CRC tumorigenesis, highlighting oncofetal H19 as the expert player in CRC biology. H19 is frequently deregulated in several tumor types and responds to stress factors such as reduced *P53* and hypoxia, leading to cancer initiation and progression. It is found that H19 is stably overexpressed in CRC tissues and its conserved secondary structure regulates protein expression or interacts with target miRNAs and their downstream genes as a ceRNA sponge, mediating CRC proliferation, expansion, EMT, intravasation into blood and lymph vessels, and extravasation to secondary sites. Furthermore, pre-clinical and clinical studies have highlighted H19 as a cancer biomarker and its therapeutic potential. Besides the current studies on the interaction between H19 with various miRNAs in CRC, EMT triggered by extracellular signaling pathways induces H19/miR-675 expression in CRC. Not surprisingly, H19/miR-675 modulates the expression of one of the major genes in EMT, E-cadherin, where H19 suppresses E-cadherin. Loss of E-cadherin is universally regarded as the hallmark of the EMT process. Despite enormous breakthroughs in understanding the role of H19/miR-675 in CRC development, many important aspects still need to be addressed. Although it is known that H19 can act independently of miR-675, in the EMT process, where H19 and miR-675 act together, it is not clear whether the lncRNA and miRNA produce a synergistic effect, have complementary roles, or cancer-type specific modes of activation, in which activation of either one can lead to different phenotypic outcomes of EMT.

It is also required to understand what triggers CRC cells to use H19, miR-675, or both in the metastatic cascade. H19 is the precursor of miR-675; taking into consideration that miR-675 is processed at the expense of H19, an additional mechanism of miR-675 processing is possible since both are upregulated in CRC. However, further investigation is needed to know if miR-675 has its regulatory sequences or mechanisms. Moreover, elucidation is required on molecular or environmental mechanisms governing the recruitment of H19/miR-675 during EMT in CRC. Liquid biopsy represents a novel non-invasive approach that holds great promise to diagnose cancer. Future research about non-invasive analysis on quantification of circulating levels of H19 in CRC patients is essential to identify if serum exosomal H19/miR-675 would serve as a promising liquid biopsy biomarker for large-scale diagnosis and prognosis of CRC.

H19 has been extensively studied in the context of CRC. While there is evidence to suggest its oncogenic role and potential as a therapeutic target or biomarker, ongoing research is necessary to unravel the complexities of its functions and precise contributions to CRC together with its miRNA transcript, miR-675. Even so, some of the questions that are yet to be addressed include:

- What is the precise function of H19/miR-675 in the colonization of secondary sites? Does the H19/miR-675 axis play a part in determining whether differentiation occurs at the secondary site?
- How does H19/miR-675 contribute to the process of multidrug resistance induced by EMT? Is this process influenced by the capacity of H19/miR-675 to modulate the transcription factors responsible for EMT?
- Could the H19/miR-675 axis introduce a new molecular mechanism to explain the enduring relationship between invasiveness and drug resistance?
- H19 is expressed not only in cancerous cells but also in neighboring stromal cells. What role does H19 expression in the stroma play in the context of the metastatic process?

Lastly, the field of lncRNA research continues to evolve, and further insights into H19's role in CRC are likely to emerge, including substantial and broad-scale research to thoroughly explore the diagnostic and prognostic potential of H19/miR-675 in the context of cancer progression, resistance, and relapse.

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