

# Regulation Mechanism of p-Akt and lncRNA H19 in Colorectal Cancer

Wenhan Yin<sup>1\*</sup>

<sup>1</sup>Guangzhou Dublin International College of Life Science and Technology, South China Agricultural University, Guangzhou, Guangdong, China

**Abstract.** Colorectal cancer (CRC) has been a widespread kind of malignant neoplasm worldwide. It has been one of the major contributing reasons for cancer-related deaths globally, with high incidence and mortality; colorectal liver metastases (CRLM) are the main causes of death in CRC patients, and signaling pathway dysregulation-driven abnormal cell proliferation is the core driver of CRC progression. Clarifying the regulatory mechanisms of key molecules in proliferative pathways is critical for developing therapeutic strategies. This study focuses on investigating the abnormal activation of phosphorylated Akt (p-Akt) in the PI3K/Akt pathway during CRC proliferation and the regulatory function of long non-coding RNA (lncRNA) H19. These results show that p-Akt activation in CRC is driven by multiple mechanisms, including overactivation of upstream oncogenic pathways (e.g., HGF/c-Met), abnormal expression of phosphatases (e.g., PRL-3), inactivation of negative regulators (e.g., PTEN), and tumor microenvironment factors (e.g., exosomes). lncRNA H19 is obviously overexpressed in CRC tissues, promoting cell proliferation via ceRNA-mediated mechanisms (e.g., adsorbing miR-200a to upregulate  $\beta$ -catenin). Notably, H19 regulates p-Akt activation through ceRNA mechanisms to target PI3K/Akt pathway mRNA and protein interactions (e.g., binding to hnRNPA2B1), forming a regulatory network that drives CRC progression. This paper systematically integrates the regulatory relationship between H19 and p-Akt, providing a comprehensive theoretical basis for developing combination-targeted therapies (e.g., H19 siRNA combined with PI3K inhibitors) for CRC.

## 1 Introduction

Colorectal cancer (CRC), involving the colon and rectum, is one of the most frequent and major contributing causes of cancer-related deaths across the globe. As the cancer with the third most frequent diagnosis rate globally, CRC represents a remarkable part of all new cancer cases. In addition, due to lifestyle changes such as an increased high-fat diet and reduced exercise, the occurrence rate of CRC in developing countries is rising [1]. The occurrence and development of CRC are closely related to a series of genetic variations in special types of genes, among which abnormal signaling pathways are the key cause of uncontrolled CRC cell proliferation. The pathway of phosphatidylinositol 3-kinase (PI3K) to protein kinase B (Akt) acts as an important signaling cascade. It can regulate various cellular activities like metabolism, protein synthesis, survival of the cell, and proliferation; But in CRC, this pathway is often overactivated, especially through the activation form of Akt - phosphorylated Akt (p-Akt). P-Akt is commonly present in CRC and can promote cancer cell proliferation by activating downstream molecules [2].

A lot of studies have explored the mechanism of abnormal activation of the PI3K/Akt-p-Akt pathway in CRC. Results found that this abnormal activation in

cancer is driven by multiple mechanisms, including homologous phosphatase tensin protein (PTEN) mutations and other genomic changes [2]. During recent periods, long non-coding RNAs (lncRNAs) have been proven in studies they play an important function in CRC, among which lncRNA H19 often has high expression in CRC, correlating with a poor prognosis in patients [3]. Currently, research indicates that H19 may affect the PI3K/Akt-p-Akt pathway through an endogenous competitive RNA (ceRNA) mechanism: H19 can act as a "molecular sponge" to adsorb microRNAs (miRNAs) that typically inhibit Akt synthesis. When these miRNAs are adsorbed, the production of PI3K and Akt increases, which may lead to elevated p-Akt levels [4]. However, there are still gaps in existing research: the specific binding sites of H19 to these miRNAs are not yet clear, and whether H19 can directly interact with PI3K protein to affect p-Akt also needs further verification.

Although significant progress has been made in the study of PI3K/Akt pathway and H19 in CRC, the mechanism by which H19 and p-Akt synergistically promote CRC cell proliferation remains unclear. For example, most studies have not validated whether there are differences in this interaction between different types of CRCs, such as microsatellite stable and microsatellite unstable CRCs. Meanwhile, liver

\* Corresponding author: [yinwenhan@stu.scau.edu.cn](mailto:yinwenhan@stu.scau.edu.cn)

metastasis is a major issue faced by CRC patients, but there is currently a lack of relevant research on whether H19 has different effects on p-Akt in early CRC and metastatic CRC. This review will summarize the ways in which the PI3K/Akt-p-Akt pathway is overactivated in CRC and the regulatory role of lncRNA H19, and explore in depth the specific mechanisms by which H19 affects the PI3K/Akt pathway.

## **2 P-Akt as a Marker of Tumour Invasiveness**

Akt, as a serine/threonine kinase, can be widely expressed in various kinds of cells. After Phosphorylation on specific amino acid residues, it becomes p-Akt, which can improve the survival ability, proliferation potential and invasiveness of cancer cells. Akt is one of the main downstream effector molecules mediating PI3K function, playing a significant role in the PI3K/AKT signaling pathway. This pathway represents a crucial function among many types of cancer. It regulates diverse cancer-related markers like cell survival, metastasis, and metabolism [2].

### **2.1 Activation of p-Akt in CRC**

#### *2.1.1 Overactivation of upstream signaling pathways driven by prot carcinoma genes*

In CRC, the activation of p-Akt is tightly regulated by upstream oncogenic signaling pathways. Among them, the HGF/c-Met signaling pathway is a typical inducer. In liver metastases of colorectal cancer, c-Met (hepatocyte growth factor receptor) is often overexpressed and binds to its ligand HGF, which can activate PI3K/Akt and MAPK/ERK signaling downstream. This activation directly enhances cancer cell proliferation, invasion, and metastatic capacity [1]. Besides, the process forms a positive feedback loop, further amplifying PI3K/Akt activation and driving CRC metastasis. Through JAK2/STAT3 signaling, HGF induces SOX13, which upregulates the promoters to activate c-Met expression.

#### *2.1.2 Abnormal non-precursor protein phosphatase*

Another key p-Akt regulatory factor is PRL-3 (Regenerating Liver Phosphatase 3). It is highly expressed in colorectal cancer liver metastases. PRL-3 is stabilized by the ubiquitin-specific protease. It binds to PRL-3, deubiquitinating PRL-3 to drive CRC invasion and metastasis. Studies have confirmed that PRL-3 promotes cell metastasis by activating the NF- $\kappa$ B pathway, AKT, STAT3, EGFR, IL-8 and CCL26 to regulate downstream prometastatic effector molecules [1].

#### *2.1.3 The inactivation of the pathway negative regulator*

As the most critical tumor suppressor gene mutation in colorectal cancer, PTEN inactivation serves as a core mechanism driving abnormal p-Akt activation. Under normal conditions of the PI3K-PTEN-Akt pathway, PTEN converts PIP3 into PIP2 through its lipid phosphatase function. This process inhibits excessive activation of PI3K by removing phosphate groups, prevents abnormal activation of Akt protein, and maintains the balance between PI3K/Akt pathways [5]. However, when PTEN loses activity, it can't inhibit the PI3K pathway, leading to excessive accumulation of PIP3. This ultimately converts Akt into the active form p-Akt. Persistently activated p-Akt then phosphorylates downstream substrates like GSK-3  $\beta$ , directly promoting abnormal CRC cell proliferation and tumor progression. This PTEN inactivation-induced p-Akt activation is the main reason for PI3K/Akt pathway dysfunction in CRC and also a potential target for future targeted therapies [5].

#### *2.1.4 Tumor microenvironment and exosomes in p-Akt regulation*

The tumor microenvironment (TME) is also important in regulating p-Akt activation and colorectal cancer progression. Exosomes are key mediators of intercellular communication in the TME, promoting cancer cell tumor occurrence, metastasis and treatment resistance. Akt in exosomes can promote tumor invasion. Colorectal cancer cells can secrete exosomes carrying Akt proteins that invade adjacent cells to induce epithelial-mesenchymal transition (EMT) [3]. Additionally, immune cells can also regulate through the PI3K/Akt pathway. For example, interleukin-6 (IL-6) promotes PD-L1 expression in CRC, specifically inducing phosphorylation of Akt in Cancer stem cells (CSC), promoting immune escape in CSC.

### **2.2 Functional validation of p-Akt in colorectal cancer**

#### *2.2.1 Clinical sample analysis*

Studies of clinical samples on the expression of p-Akt indicate that tumor tissues in CRC clinical specimens exhibit significantly higher p-Akt expression levels than adjacent normal tissues. Research detected that 68.3% of CRC tissues showed p-Akt positivity, much more than the positive level in adjacent tissues. This high expression is closely related to tumor size enlargement, serosal invasion, and lymph node metastasis [6]. In the study of comparison of AKT expression with 5-year survival rate in patients, patients with high p-Akt expression had a 5-year survival rate of only 38.33%, compared to 75% in negative AKT expression [6]. This indicated that p-Akt overexpression is also related to CRC progression and poor prognosis. These analyses confirmed p-Akt as a prognostic factor for CRC.

### 2.2.2 Cellular experiments

Cellular experiments validated p-Akt's role in promoting CRC cell proliferation through the PI3K/Akt pathway. Research demonstrated that Z86, a  $\beta$ -coumarin derivative targeting PI3K, could decrease p-Akt expression in colorectal cancer cells. Assays revealed slower cell proliferation rates and weakened clonogenicity, confirming that blocking the PI3K/Akt signaling pathway, thereby inhibiting p-Akt activity and suppressing tumor cell proliferation. Similar results obtained through siRNA-mediated PI3K/Akt silencing further reinforce the critical function of p-Akt in maintaining colorectal cancer cell proliferation [7].

## 3 Expression of lncRNA and its functions in colorectal cancer

### 3.1 Expression characteristics of lncRNA H19 during colorectal cancer progression

In the non-coding RNA family, lncRNAs represent one of the subgroups, identified by their length, that are more than 200 nucleotides long, as well as the absence of the ability to code for protein. Transcribed by RNA polymerase II, these molecules are divided into five different types. Among them, lncRNA H19 is one of the earliest identified types of lncRNAs, involved in processes like neurogenesis, adipocyte differentiation, cell proliferation, and programmed cell death (PCD). Its abnormal overexpression is linked to cancer development, and can serve as a promising prognostic indicator and target of therapy to treat specific cancer types. To clarify the expression of H19 in cancer cells, studies using real-time quantitative PCR (qRT-PCR) technology evaluated its levels in clinical samples as well as cell lines. These have confirmed its significantly high expression characteristics in colorectal cancers [8].

Clinical tissue samples have employed qRT-PCR in cancer tissues and cell lines to measure H19 expression levels. A series of cellular studies includes growth curve analysis, viability testing, and colony formation studies. These results revealed that in colorectal cancer tissues, H19 was expressed at a significantly higher level than in adjacent non-cancerous tissues in 30 pairs of tested samples [8]. Another study conducted qRT-PCR analysis on 20 colorectal cancer tissues, adjacent normal tissues, colorectal cancer cell lines SW480, HCT116, SW620, and normal NCM460 epithelial cells. The outcomes clearly showed that H19 expression levels in colorectal cancer tissues and cell lines were obviously higher than those in adjacent normal tissues and normal NCM460 cells [9]. Additionally, qRT-PCR revealed that the long non-coding RNA H19 was significantly upregulated in both the primary period of tumors and the metastatic period of tissues [1]. Overall, extensive research indicates that H19 is greatly expressed within colorectal cancer tissues and various cell lines, suggesting its close association with the developmental process and progression of colorectal cancer.

### 3.2 Functional roles of lncRNA H19 on colorectal cancer cell proliferation

Multiple studies have investigated the influences of H19 overexpression/silencing on colorectal cancer cell proliferation through functional experiments. Researchers found that in colorectal cancer, H19 can work like a "molecular sponge" to combine with miR-200a in CRC cells. This interaction inhibits the activity of miR-200a, preventing it from silencing the downstream target gene  $\beta$ -catenin. Thus, the expression of the  $\beta$ -catenin gene increases, promoting colorectal cancer cell proliferation [8]. These mechanisms demonstrate how lncRNA H19 drives tumor progression through this regulatory pathway. Another study transduced H19 siRNA and a negative control sequence into SW620 colorectal cancer cells, via flow cytometry, colony formation assays, and the CCK8 method to assess proliferation. Results showed that silencing H19 expression (si-H19 group) markedly decreased the survival rate and quantity of SW620 cell clones [9]. These findings indicate that H19 regulates colorectal cancer cell proliferation and has important functions in this process, with its high expression status favoring tumor progression.

## 4 Correlation analysis between lncRNA H19 and p-Akt in colorectal cancer

### 4.1 Correlation analysis between H19 and p-Akt in clinical samples of colorectal cancer

The correlation between clinical sample expressions is crucial for revealing molecular functional associations. Numerous studies have confirmed that lncRNAs and the PI3K/AKT signaling pathway have a significant effect during the process of cancer, particularly in controlling biological activities like cell proliferation, invasion and metastasis, and angiogenesis. Current researches indicate that lncRNAs can influence cellular functional states through direct or indirect interactions with the PI3K/AKT signaling pathway, thereby exerting an effect on cancer progression [10]. During recent times, more and more investigations have identified lncRNAs as well as the PI3K/AKT signaling pathway as viable candidates for biomarkers, which can be used in the diagnosis, treatment, and prognosis prediction of tumors originating from the digestive system [10].

Studies show that in the development of cancer, long non-coding RNA H19 plays a important oncogenic role. Its mechanisms are closely associated with the competitive endogenous RNA (ceRNA) effect and the PI3K-Akt signaling pathway. H19 can work as a regulatory factor through the ceRNA mode. It competitively binds to microRNAs (miRNAs), releasing their inhibitory effects on downstream target mRNA. Ultimately, upregulating the expression levels of various cancer-related mRNA. Research data indicate that target mRNA modulated by H19 (like AKT3, CSF1, MET, COL1A1) are functionally concentrated within the PI3K-Akt signaling pathway. The regulation significantly impacts various molecular activities tightly

associated with the PI3K-Akt signaling pathway. These combined changes drive cancer progression [4]. Further studies indicated that high H19 expression in patients was related to poorer prognosis, suggesting that H19 can modulate p-Akt pathway, thereby involving in the progression of CRC [4].

#### **4.2 Validation of H19 regulation of key PI3K/Akt pathway molecules through the ceRNA mechanism**

The ceRNA mechanism refers to lncRNAs that control the expression of genes by combining with microRNAs (miRNAs), releasing miRNAs from their inhibitory effects on target genes. In colorectal cancer, H19 has been confirmed to regulate key molecules in the PI3K/Akt pathway through this mechanism. Researchers analyze bioinformation to prove interaction between lncRNA-miRNA and miRNA-mRNA, establishing lncRNA/pseudogen-miRNA-mRNA ceRNA network [4]. They also found that the mRNA in the ceRNA network was highly associated with the PI3K signaling pathway. Assay of enrichment mRNA in the ceRNA network showed that these mRNA were involved in 11 KEGG pathways, which are closely linked to biological processes, including the PI3K-Akt signaling pathway [4]. Functionally, knocking down H19 or inhibiting related miRNA activity leads to reduced expression of key PI3K/Akt pathway molecules, decreased p-Akt activity, and suppression of cell proliferation, migration, and invasiveness.

#### **4.3 Detection of H19-interacting proteins involved in p-Akt activation**

Interactions between H19 and related proteins also play important roles in regulating p-Akt activation. A study identified a direct binding between H19 and hnRNPA2B1, which was verified using RNA pull-down and RNA immunoprecipitation (RIP) assays [3]. As an RNA-binding protein, hnRNPA2B1 has been shown to foster tumor initiation and progression. In CRC, the binding between H19 to hnRNPA2B1 promotes the mutual influence between hnRNPA2B1 and molecules such as RAF-1, activating the RAF-ERK pathway and resulting in the induction of EMT. It has an important function in tumor invasion and metastasis, thereby promoting the migration, invasion and metastasis of colorectal cancer cells [3].

Knockdown of hnRNPA2B1 inhibited H19-induced p-Akt activation and cell migration. Study found that knockdown of HNRNPA2B1 promotes the retention of exon 11, which reduces the abundance of RON  $\Delta$  165 isoform. Subsequently, the decrease in RON  $\Delta$  165 levels inhibits the activation of the Akt/PKB signaling cascade. When the Akt/PKB signal is suppressed, the expression level of epithelial cell markers is upregulated and stromal cell markers (wave protein) are downregulated, ultimately inhibiting the EMT process.

## **5 Conclusion**

This paper systematically explores the abnormal activation of p-Akt in the PI3K/Akt pathway during CRC cell proliferation, and the specific regulatory mechanisms mediated by lncRNA H19. Comprehensive literature reviews indicate that p-Akt, as the active form of Akt, is dysregulated in CRC through major mechanisms. Overactivation of upstream oncogenic signaling pathways (e.g., HGF/c-Met pathway) forms positive feedback loops that enhance p-Akt activation levels, promoting CRC cell proliferation and metastatic potential. Stabilized PRL-3 regulates downstream effector factors, driving p-Akt-mediated invasion processes. Third, inactivation of the tumor suppressor PTEN can lead to excessive PIP3 accumulation and continuous activation of p-Akt. Tumor microenvironment components such as exosomes and immune cells secreting IL-6 further regulate p-Akt activity, driving immune evasion. Clinically, p-Akt overexpression is associated with tumor size increase, lymph node metastasis, and reduced 5-year survival rates. Cell experiments have confirmed that PI3K/Akt inhibitors can suppress CRC proliferation by reducing p-Akt levels. Meanwhile, the long-chain non-coding RNA H19 is persistently overexpressed in colorectal cancer tissues. This molecule promotes cell proliferation through two mechanisms, using ceRNA activity and interacting with proteins to activate p-Akt. This establishes an H19-p-Akt regulatory network that promotes colorectal cancer progression. This paper summarizes evidence to clarify the regulatory relationship between H19 and p-Akt, laying the theoretical foundation for developing p-Akt/H19 prognostic biomarkers and designing combination therapies. The key limitations exist as the current study is based on existing literature and lacks experimental validation of unreported regulatory interactions. Future studies should identify new regulatory molecules in this pathway through in vitro and in vivo studies, while validating H19/p-Akt-targeted therapies to advance precise medicine development for colorectal cancer.

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