H19: an example of lncRNA-mediated chemoresistance

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Colorectal Cancer and Chemotherapy

Colorectal cancer (CRC) is the third most prevalent cancer type and the third leading cause of cancer related deaths worldwide.

Adjuvant chemotherapy has been demonstrated to extend life expectancy and improve the possibility of cure in patients with advanced colorectal cancer.

However, most colorectal cancers remain unresponsive to chemotherapy and thus it is still a life-threatening disease and a big challenge to the clinics.

Drug resistance acquisition is one of the main issues in chemotherapy

- Intrinsic Chemoresistance
- Acquired Chemoresistance
A diverse range of anatomic and molecular mechanisms have been implicated in drug resistance.

**Pharmacokinetic Resistance**

**Physics of the tumor site**

**Pharmacodynamic Resistance**
Mechanisms of Chemoresistance

Drug transport and metabolism

Drug Efflux: Several cell membrane transporter proteins promoting drug efflux.

Drug activation and inactivation: drug inactivation or lack of activation.

Alterations in drug targets

Change in expression of Target: Genetic or epigenetic mutations that causing the over-expression of target molecules.

Change in binding domain of Target: Mutation in specific target molecules that don’t block their activity.

Metabolic conversion of capecitabine to FU.
Koukourakis G. V. et al, 2008

MDR1 mediated chemoresistance.
Cornelison R. et al, 2017
Mechanisms of Chemoresistance

Dysfunction of DNA damage repair
Chemotherapeutic drugs induce DNA damage which may contribute to the acquisition of cytotoxicity. Cancer cells tend to be more resistant as a result of abnormal DNA damage by misregulation of DNA repair and DDR pathways.

Downstream resistance mechanisms

Downregulation of Apoptosis: Genetic/Epigenetic mutations causing overexpression of anti-apoptotic proteins or TFs that controls apoptotic pathways and downexpression of pro-apoptotic proteins.

Autophagy: Can facilitate cancer cell survival during metabolic stresses caused by anticancer agents.
LncRNA expression is widely altered in cancers and that lncRNAs participate in various aspects of tumorigenesis:

Cell proliferation, Apoptosis, Migration and Invasion, **Drug Resistance**.

**What are LncRNAs?**

LncRNAs include different types of RNA polymerase II (Pol II)- transcribed molecules with sizes **over 200 nt** in length.

There are an estimated abundance of 5,400 to **more than 10,000 lncRNAs transcripts in humans**. They often harbor a poly-A tail and can be spliced, similar to mRNAs.

It has been shown that **lncRNAs functions depend on their subcellular location**: a wide range of subcellular localization patterns, including nucleus, cytoplasm and both.
LncRNAs: Functions

Roles in nuclear structures: they help to the structure of nuclear speckles, paraspeckles, and interchromatin granules.

Roles in regulation of gene expression at transcriptional and post-transcriptional levels in multiple biological processes and cellular contexts.
LncRNAs are involved in cancer cell drug resistance with different regulated targets/pathways.
LncRNA H19

H19 gene is located in 11p15.5 downstream the insulin-like growth factor 2 (Igf2) gene and encodes for a 2,3kb capped, spliced and polyadenylated RNA.

H19 is highly expressed from the onset of embryogenesis to fetal life in vital organs such as the fetal adrenal, liver, and placenta but is downregulated in postnatal stages.

Plays pivotal roles in embryonal development and growth regulation

Structure of IGF2/H19 Locus.
Huang Z, Murphy SK.
**Imprinting regulation of H19 Expression**

Located in an imprinted region of chromosome 11.

**H19** and **Igf2** are oppositely imprinted and co-expressed embryonic development suggesting a common mechanism of regulation.

**Important regulation elements**

**ICR1** is important to the imprinting state of both H19 and Igf2 and is rich in CpG residues.

Two downstream **enhancers** where open in chromatin structure on both parental alleles and could work on either Igf2 or H19.

**CAGCCC motifs** in ICR1 that binds **CTCF proteins**.

Regulation of H19/IGF2 Imprinting.
Nordin M et al, 2014
Enhancers could work on either Igf2 or H19 but:

The methylation of the paternal H19 by **DNMT** inhibits his expression and thus gives enhancers a chance to work on the Igf2 gene.

**ICR1** and the H19 promoter are unmethylated on the maternal chromosome, leading to transcription of maternal H19.

The binding of **CTCF** to CAGCC unmethylated motif in ICR1 in maternal chromosome prevent downstream enhancers from accessing Igf2.

*Regulation of H19/IGF2 Imprinting.*
Nordin M et al, 2014
**LncRNA H19 in Cancer**

H19 can act as either an oncogene or tumour suppressor: the role of H19 may differ at different times in life, or display a cell dependent and/or tumour type-dependent function.

H19 is upregulated in several cancers as, esophageal cancer, hepatocellular carcinoma, ovarian cancer, bladder cancer, breast cancer and colorectal cancer.

It is implicated in cancer progression processes such as metastasis (enhances EMT), proliferation and drug resistance. In particular in CRC it can led to Methotrexate or 5-Fu resistance.

H19 is the lncRNA with the most substantial correlation to CRC patient survival, serving as an independent predictor for OS and disease-free survival (DFS).

This lncRNA has been related with poor prognosis.
We find LOI in many types of cancer.

There are evidence that H19 is upregulated in many types of cancer.

In several types of cancer we can found LOI as the cause of the overexpression of H19.

In colorectal cancer we don’t found LOI in H19 ICR, however is overexpressed.
H19 expression in tumors influences several hallmarks of cancer

Promotes epithelial to mesenchymal transition in colorectal cancer via **Wnt/β-catenin** pathway.

Promotes the migration and invasion of colon cancer cells via **MAPK** signaling pathway.

Promotes chemoresistance against several chemotherapeutics like **methotrexate** and **5-FU**.
Chemoresistance mediated by H19 in tumors

H19 mediates methotrexate chemoresistance via **Wnt/β-catenin** pathway.

**Methotrexate** inhibits dihydrofolate reductase (DHFR).

Knockdown of H19 improves the sensitivity to methotrexate of resistant cells.

Wu KF et al., 2017
Does H19 play a role in colorectal cancer 5-Fu chemoresistance?

Often colorectal cancer is resistant to 5-Fu therapy.

It is necessary to find new targets for the treatment of CRC 5-Fu resistant cases.

Therapy-induced autophagy is a mechanism of resistance to anticancer agents.

H19 promotes 5-Fu resistance in other types of tumor.
5-Fu based chemotherapy

5-Fu is a nucleoside analog inhibitor. Is a pyrimidine analog.

It blocks deoxythymidylate synthesis acting as a competitive inhibitor in thymidylate synthase.

It is used in chemotherapy for many types of cancer.

Often colorectal cancer is resistant to 5-Fu therapy.

\[
\begin{align*}
5\text{-Fu} + \text{PRPP} &\rightarrow 5\text{F-UMP} + \text{PP} \\
\text{Uracil phosphoribosyltransferase} \text{ (it also actives 5-Fu)} &
\end{align*}
\]

Next 5F-UMP must be converted to fluorodeoxyuridilate.
H19 is correlated with colorectal cancer recurrence

H19 indicates a poor prognosis of colorectal cancer.

H19 is an independent prognosis factor for CRC aggressiveness.

Expression of H19 could be significant to predict a clinical outcome.

H19 play a role in colorectal cancer recurrence
**H19 increases the cells chemoresistance to 5-Fu**

Creation and identification of cell lines 5-Fu resistant.

**H19** is more expressed in cell lines 5-Fu resistant.

In drug sensitive cells, **H19** is induced by treatment with 5-Fu.

**Expression of H19 is correlated with cells 5-Fu chemoresistance**
Overexpression and knockdown of H19 was performed to see the effect on 5-Fu chemoresistance.

H19 increases IC50 value of 5-Fu in cell cultures.

H19 permits cells to survive in higher concentration of 5-Fu
H19 influences apoptosis in presence of 5-Fu

Knock down H19 cell lines increases cell apoptosis when treated with 5-Fu.

After treatment with 5-Fu levels of cleaved PARP and Caspase-3 increases.

H19 enhances resistance to 5-Fu via reducing cell death only under 5-Fu stress
H19 induces cancer chemoresistance via the autophagy pathway.

Western blotting showed that H19 significantly upregulated the formation of autophagy marker LC3-II.
H19 induces cancer chemoresistance via the autophagy pathway

Transmission electron microscopy showed an increase in the formation of autophagic vesicles in the H19 overexpressed HCT8 cells, and lesser autophagic vesicles in the H19 knocked down HCT8Fu cells.
**H19 induces cancer chemoresistance via the autophagy pathway**

Addition of CQ could block the autophagic flux in the cells.

H19-induced 5-Fu resistance was abolished by CQ, which was confirmed by:

**MTT assay**
H19 triggers autophagy via SIRT1

Western blotting was performed to analyze SIRT1 expression status in pcDNA-H19 transfected CRC cells.

Results showed that only the expression of SIRT1 was upregulated after pcDNA-H19 transfected both in HCT8 and HCT116 cells.
H19 triggers autophagy via SIRT1

Western blotting was used to detect LC-3I, LC-3II, p62, and SIRT1 expression in HCT8 and HCT116 cells transfected with pcDNA-H19 and/or si-SIRT1.
Overexpression of H19 dramatically induced LC3 aggregation and increased the autophagosomes in CRC cells, which was markedly attenuated by the silence of SIRT1. All the above data indicated that H19 induces autophagy of CRC cells via upregulating the expression of SIRT1.
H19 triggers autophagy via SIRT1

Effects of SIRT1 on H19-induced 5-Fu resistance

observed that SIRT1 knockdown blocked the H19-induced CRC cell 5-Fu resistance.


**H19 triggers autophagy via SIRT1**

The *expression of SIRT1* was detected by *immunohistochemical analysis*.

Results showed that *SIRT1* protein was *located in both cytosol and nuclei* of CRC cells.

Expression of SIRT1 was analyzed by IHC in the CRC tissues and paired adjacent normal samples.
H19 triggers autophagy via SIRT1

Recurrent CRC tissues from patients with recurrence had stronger SIRT1 expression compared with that of non-recurrence CRC tumor tissues.

This phenomenon indicated that SIRT1 had underlying association with 5-Fu chemoresistance in CRC.

IHC analysis was performed to determine the SIRT1 staining scores in CRC tissues with distinct recurrence status.
H19 triggers autophagy via SIRT1

To determine whether SIRT1 was a potential target gene of H19, they transfected cells with pcDNA-H19 or si-H19 and then detected the expression of SIRT1. H19 overexpression led to SIRT1 mRNA and protein levels increase at 72 h post transfection, and vice versa.
**H19 sponges miR-194–5p as ceRNA**

H19 is included in micro-RNA ribonucleoprotein complex (miRNP), probably through binding with miR-194–5p.

Luciferase and RIP analysis confirmed the binding of H19 to miR-194-5p.

Results of the reversal experiment showed that while H19 down-regulation led to decreased expression of SIRT1, simultaneous miR-194-5p down-regulation was able to reverse the inhibition of SIRT1 expression in HCT8Fu resistant cell line.
H19 sponges miR-194-5p to modulate SIRT1 expression

H19 correlated with SIRT1 expression in patients

Positive correlation between H19 and SIRT1 expression in CRC tissues.

These results confirmed that H19 abolishes the miR-194-5p-mediated repressive activity on SIRT1 by competitively binding miR-194-5p.
H19 sponges miR-194-5p to modulate SIRT1 expression

The functional model underlying the mechanism of H19 on tumor chemoresistance
CONCLUSION

- H19 is upregulated in CRC recurrence samples appears to be a potential biomarker for predicting 5-Fu chemoresistance.

- SIRT1 dependent autophagy pathway could affect 5-Fu chemoresistance in colon cancer cells, which was modulated by H19/miR-194-5p axis.

- As the amount of H19 is associated with the risk of CRC recurrence, the measurement of H19 post-surgery may be an effective approach to predict patients’ outcome.
Are conventional chemotherapeutic regimens including 5-Fu suitable for CRC patients with a high amount of H19?

CRC patients with a high amount of H19 may be treated with conventional chemotherapy in combination with anti-H19 treatment and/or an autophagy inhibitor.
Bibliography


THANKS FOR YOUR ATTENTION