

Role of long non-coding RNA in cells: Example of the *H19/IGF2* locus

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ABSTRACT

In the past decade, studies of non-coding RNAs increase. Non-coding RNAs are divided in two classes: small and long non-coding RNA. It was shown that long non-coding RNAs regulate expression of 70% of genes. Long non-coding RNAs are involved in several cellular processes like epigenetic regulation, dosage compensation, alternative splicing and stem cells maintenance for example. Misregulations of their expression induce diseases such as developmental syndrome or cancer. In this review, we describe some functions of long non-coding RNA in cells. Furthermore, we study the *H19/IGF2* cluster: an imprinted genomic locus located on chromosome 11p15.5. Genomic imprinting allows gene expression from a single allele in a parent-origin-dependent manner. This cluster encode for the first long non-coding RNA identified: *H19*. In 1990, it was established that *H19* functions as a *riboregulator*. Recently, it was shown that *H19* is a precursor of microRNA (*hsa-miR-675*), and several news transcripts were identified at the *H19/IGF2* locus. So, the complexity of this locus increasing, in this review, we summarize our current understanding about the *H19/IGF2* cluster both in terms of transcription as well as in terms of functions in cells. We highlight the involvement of *H19*, its new antisense transcript *91H* and its microRNA, in the regulation of IGF receptor function and in cell cycle progression.

Keywords: *H19* Gene; Genomic Imprinting; Non-Coding RNA; Cell Cycle

1. INTRODUCTION

Recently, the ENCODE project have proved that approximately 90% of the genome is transcribed. In cells, about 2% of sequences coding for protein, the others

transcripts act as introns or non-coding RNA. These non-coding transcripts are composed of non-protein coding gene and background of transcription. This transcriptional noise would allow maintaining chromatin in a conformation favourable to the transcription of protein-coding gene. In the past decade, studies of non-protein coding gene, also called non-coding RNAs (ncRNAs), have increased and revealed that they are important in cells. ncRNAs are highly regulated and have several functions for normal development of tissues to tumorigenesis. Non-coding RNAs are divided in two classes of RNAs: the small and the long non-coding RNAs.

Small non-coding RNA (<200 nts), notably micro-RNAs (miRNAs), are involved in post-transcriptional regulation of RNA. MiRNAs are the most studied of the small ncRNAs because they regulate 60% of protein-coding gene. Mostly, they are encoded by the genome, transcribed by RNA polymerase II, cleaved by Drosha in the nucleus and by Dicer in the cytosol. Then, they interact with Argonaut protein family to form the RISC complex (RNA-induced silencing complex). This complex allows the function of miRNA [1]. They induce silencing of gene by degrading or inhibiting the translation of mRNA into protein. They are involved in all process of the cell: proliferation, cell cycle progression, differentiation, and apoptosis. Two others small non-coding RNAs are described: piRNA (PIWI-interacting RNA) and snoRNA (small nucleolar RNAs). piRNAs have recently been discovered. They are important for germline development and for the suppression of transposon activity in germline cells. SnoRNA are components of ribonucleoproteins (snoRNPs). They are responsible for post-transcriptional modifications of rRNA that take place in the nucleolus (the nucleus compartment where ribosomes are formed) [1].

Long non-coding RNAs (LncRNAs (>200 nts)) can have different origins: mRNA, antisense transcripts or long-intergenic non-coding RNA (lincRNA). LincRNAs are produced by sequence between two genes or set of

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genes. In *Homo sapiens*, 4500 lincRNAs have been discovered but only 108 lincRNAs are studied and published [2]. LincRNAs have an important place in cell as they regulate expression of 70% of genes. They are involved, for example, in epigenetic regulation, alternative splice or stem cells maintenance. Misregulations of their expression induce diseases such as cancer.

The first imprinting lincRNA identified is the *H19* RNA. *H19* is transcript from the *H19/IGF2* cluster located on the chromosome 11p15.5. Recently, the complexity of this locus has increased since several new transcripts were identified.

In this review, firstly, we describe some functions of long non-coding RNA in cells. Then, to illustrate our point, we summarize our current understanding about the *H19/IGF2* cluster, its complexity and its function in cells.

2. LONG NON-CODING RNA FUNCTIONS

2.1. Dosage Compensation

The lincRNA *Xist* (X-inactive-specific-transcript), an lincRNA of 17,000 nucleotides, is the most studied. This lincRNA is implicated in the X chromosome inactivation in dosage compensation [3]. *Xist* is encoded by the X chromosome and acts in *cis*. Thanks to its conserved repeat motif RepA, *Xist* interacts with the Polycomb repressive complex (PRC2), the complex responsible for trimethylation of histone H3 at Lys²⁷, and targets this complex to the XIC (X-inactivation centre). PRC2 complex induces histone modifications, heterochromatin formation and silencing of the targeted X chromosome. *Xist* is regulated by two other lincRNAs, one acting negatively *Tsix*, and the other positively *Jpg*. *Tsix*, antisense RNA to *Xist*, is expressed from the X-active chromosome and inhibits *Xist* expression in *cis*. When expressed, *Tsix* recruits DNA methyltransferases (Dnmt3a) to repress the expression of *Xist*, and blocks the interaction between *Xist* and the PRC2 complex.

2.2. Epigenetic Regulation

The lincRNA *Air* is submitted to the genomic imprinting. It consists of a 108 kb-long transcript. *Air* promoter is localized in the imprinting centre within the *IGF2r* gene and it is necessary for the paternal repression of the gene of the locus [4,5]. However, the molecular mechanism remains unclear and authors propose a hypothesis of methylation propagation from the *IGF2r* gene or of repressive ARN/protein complexes formation.

The third lincRNA well studied is located in the cluster *Kcnq1/Kcnq1ot1* on the chromosome 11 in position 15.5. *Kcnq1ot1* RNA is a 91 kb transcript which is expressed in antisense orientation from a highly conserved and differentially methylated region *Kcnq1* ICR or ICR2 pre-

sent in intron 10 of *Kcnq1* gene. Expression of this transcript is exclusively paternal. Indeed, the *Kcnq1ot1* promoter shows a maternal specific methylation. This differential epigenetic mark is lost in patients affected by Beckwith-Wiedemann syndrome with RNA biallelic expression [6-8]. More recently, Pandey and colleagues (2004) have documented that the *Kcnq1ot1* transcript has a key role in silencing of genes contained in the *Kcnq1* gene imprinted region and that it participates directly or indirectly to the methylation but without RNA interference mechanisms [9]. Furthermore, interruption of *Kcnq1ot1* RNA production by the insertion of a polyadenylation sequence downstream of the promoter also caused a loss of both silencing activity and methylation spreading. Thus, the antisense RNA plays a key role in the silencing function of the ICR [10].

2.3. Regulation of Alternative Splicing

The lincRNA *Malat1* (Metastasis-associated lung adenocarcinoma transcript 1), also known as *NEAT2* (Nuclear-enriched abundant transcript 2), is polyadenylated and overexpressed in various cancers. It is a conserved transcript among mammals of 6 - 7 kb, localized in nuclear. RNA-fish studies have shown that *Malat1* is localized in sub-compartment of nuclear: nuclear speckles [11, 12]. Contrary to *NEAT1* (a lincRNA essential for nuclear paraspeckle formation), *Malat1* is not essential to nuclear speckle integrity. This compartment is composed in majority of factors involved in pre-mRNA splicing, like SR family protein and protein implicated in RNA transport for example. Bernard *et al.*, have shown that *Malat1* controls of SR family protein (SF2/ASF) of splicing factor to transcription site. Tripathi and co-workers have established that *Malat1* regulates expression levels, localization and activity of SR protein. Targets genes of *Malat1* are tissue-dependent. In neuronal cells, *Malat1* regulates preferentially splicing of genes involved in synaptogenesis like Neurologin gene (*Nlgn1*) and synaptic cell adhesion molecule 1 (*SynCAM1*).

2.4. Stem Cells Maintenance

Recently, it was observed that 133 lincRNAs were overexpressed and 104 down-regulated in ESC (Embryonic Stem Cell) or iPSC (induced Pluripotent Stem Cells) compared with fibroblast [13]. They have shown that twenty-eight lincRNAs upregulated in iPSC, notably lincRNA-RoR, could be regulated by pluripotency transcription factors OCT4, SOX2 or NANOG. Depletion of lincRNA-RoR inhibits iPSC colony formation. They have proved that lincRNA-RoR promotes survival of iPSC and ESC by preventing the activation of stress pathways like p53 response. This RNA is important to reprogramming stem cells whence its name "Regulator

of Reprogramming". So, they identified the first functional lincRNA in establishing iPSC.

Furthermore, a study has identified several ncRNAs implicated in stem cells differentiation [14]. They have demonstrated that lincRNAs are associated with trimethylated H3K4 histones and histone methyltransferase MLL1. These suggest that lincRNAs have a role in epigenetic regulation during ES cell differentiation.

2.5. LincRNA in Cancer

In human, HOX transcription factor are encoded by four HOX cluster on four different chromosomes: HOXA to D. From HOX cluster only 39 transcription factors are expressed but 231 ncRNAs are transcript [15,16]. The well HOX ncRNA studied is *HOTAIR* (Hox antisense transcript RNA). It is antisense RNA of 2.2 kb, transcript from the HOXC cluster. Studies have shown that *HOTAIR* regulates expression of genes on HOXD cluster, so acts in *trans*. Indeed, when expressed, due to its 5' domain, *HOTAIR* interacts with PRC2 complex, notably Suz12 and EZH2 protein [15]. PRC2 complex induces trimethylation of Histone H3 lysine 27 on HOXD cluster (an inactive methylation). *HOTAIR* can also interact with the LSD1/CoREST/REST complex: a complex involved in trimethylation of Histone H3 lysine 4 (active chromatin). *HOTAIR* regulates chromatin conformation from active chromatin to inactive. So, it is scaffold RNA [17]. In several cancers, notably breast cancer, *HOTAIR* expression is associated to metastasis [18]. Authors have shown that *HOTAIR* overexpression increases cells invasion and metastasis in mice. They established that *HOTAIR* invasion is PRC2 complex dependent. So, *HOTAIR* expression is associated to poor prognosis.

LincRNAs are implicated in several cellular processes (epigenetic regulation, dosage compensation, stem cells self-renewal and differentiation). In some cases, lincRNA expression allows maintains of stem cells pool for example, whereas, sometimes, lincRNA expression is responsible of cancers. So, expression of lincRNA must be well regulated. They can act *per se* but can also acts as precursor of small ncRNA such as microRNA. The lincRNA *H19*, the first imprinting ncRNA discover is the precursor of the microRNA: miR-675 [19]. Recently, several groups have identified targets of this miRNA in several cell lines [20,21]. To illustrate our point, we summarize our current understanding about the *H19/IGF2* cluster, its transcription complexity and its function in cells.

3. THE *H19/IGF2* CLUSTER

3.1. Genomic Imprinting at the *H19/IGF2* Cluster

The *H19/IGF2* cluster is submitted to genomic imprint-

ing. Genomic imprinting is a form of epigenetic gene regulation that results in expression of a single allele in a parent-of-origin-dependent manner. This form of monoallelic expression is essential for normal development. Despite extensive studies, the molecular mechanisms of genomic imprinting remain unclear. However, some hallmarks of this phenomenon have been identified and we can note that:

- Gene expression is allele-specific and tissue or stage-specific.
- Many of imprinted genes are found in clusters throughout the genome. The clusters contain two or more imprinted genes over a region that can span 1 Mb or more.
- Within each cluster, a common regulating region which are called "imprinting control region" (ICR, also called IC for imprinting Centre or ICE for imprinting control element) controls the imprinting of all genes in the cluster and can act over hundreds of kilobases. ICRs are designed as differentially methylated regions with parental-specific modifications that determine their activity. Deletions of this region lead to the loss of imprinting of multiple genes of the cluster [22,23].
- More recently, it has been reported that non-coding RNA were associated with imprinted clusters and have an essential role in regulating gene expression.

The *H19/IGF2* cluster is located on the human chromosome 11 in position p15.5. This 1Mbp domain contains 9 imprinted genes and 2 independent imprinting center. The first imprinting center (ICR) regulates the cluster *H19/IGF2* and the second (ICR2), the cluster *Kcnq1/Kcnq1ot1*.

The *H19* gene is one of the first genes proven to be imprinted. This gene is co-regulated negatively with the *IGF2* gene located 200kb upstream of the transcription site of the *H19* gene. Indeed, *H19* is expressed only from the maternal allele whereas *IGF2* is expressed from the paternal allele [24]. The paternal allele exhibits several characteristics that explain the silencing of the *H19* gene: it is hypermethylated in the promoter region and the promoter shows a compact chromatin structure [25,26]. Moreover, the histone acetylation rate is lower than the one of the maternal allele [27].

Surprisingly, the *IGF2* promoter region is not methylated and its chromatin structure is favourable to a biallelic transcription [28]. However, two other differentially methylated regions (DMR) on the expressed paternal allele have been identified within the gene: the DMR1 located 3 kbp upstream the P1 promoter acts as a silencer on the maternal allele when it is unmethylated, and the DMR2, located within exons 5 and 6 is an activator on the paternal allele when it is methylated [29-31].

However, DNA methylation is not sufficient to explain

the mono-allelic expression. Indeed, the ICR is the key of the genomic imprinting: it controls the chromatin structure and regulates the effect of enhancers located downstream of the *H19* gene [32,33]. This region is located 2 to 4 kbp upstream of the transcription site of the *H19* gene. In human, it contains seven binding site of zinc-finger protein named CTCF (CCTC-binding factor) but only the sixth is differentially methylated [34]. On the maternal allele, the CTCF protein interacts with non-methylated ICR due to four consensus site (**Figure 1**) [35]. On the ICR, this protein has a chromatin insulator function as it prevents the action of enhancers on the promoter of *IGF2*. On the paternal allele, methylation of the ICR represses the *H19* expression and prevents the attachment of the CTCF protein [36]. So enhancers can activate the *IGF2* expression from this allele. Thus, *H19* is expressed from the maternal allele and *IGF2* from the paternal allele (**Figure 1**).

Chromosome conformation capture (3C) analysis shows interaction between different chromosomal regions and suggests that the CTCF protein has a critical role in the epigenetic regulation of the cluster *H19/IGF2*. Kurukuti and al. 2006 demonstrated that on the maternal allele, CTCF interact with the DMR1 and the Matrix Attachment Region (MAR3) at the *IGF2* locus to generate a tight loop around the *IGF2* gene [37,38]. This interaction creates an inactive domain where *IGF2* is far away

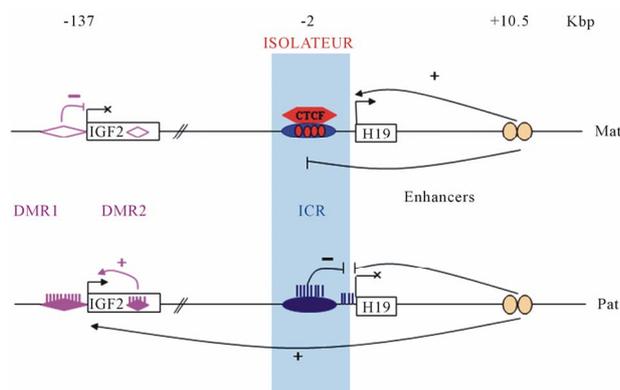


Figure 1. Genomic imprinting at the *H19/IGF2* locus. Activation of gene transcription is represented by (+), repression by (-) and inhibition of enhancers function by (\rightarrow). Relative positions are expressed in kilobase pairs relatively to the *H19* transcription start site. Gene expression is regulated by three mechanisms: methylation, enhancers activity and insulator activity. Three DNA region are differentially methylated: DMR 1 and 2 of the *IGF2* gene (violet diamond) and ICR (blue oval). On the maternal allele, the CTCF protein interacts with the non-methylated ICR (blue oval). This interaction prohibits enhancers access to the *IGF2* gene. Furthermore, the DMR1 non-methylated of *IGF2* gene acts as a silencer. On the paternal allele, the methylated ICR repress *H19* and inhibits CTCF interaction. So, enhancers can activate *IGF2* transcription. Moreover, the methylated DMR2 of *IGF2* gene activate also *IGF2* transcription.

from the enhancers. Therefore, this gene is in inactive domain so it cannot be expressed from this allele (**Figure 2**).

On the paternal allele, the methylated ICR interacts with methylated *IGF2* DMR2 moving *IGF2* into the active chromatin domain [39].

So genomic imprinting of the *H19/IGF2* cluster is allowed by DNA methylation, chromatin composition, organization and conformation.

3.2. News Transcripts at the *H19/IGF2* Locus

In 1991, an antisense transcript of the *IGF2* gene in chicken was identified [40]. Others studies have identified antisense *IGF2* transcripts of 3-4 kb in mouse and human (**Figure 3**) [41,42]. This transcript is expressed only from paternal allele and no open reading frame (ORF) was identified. Its function remains unclear, but it is a good marker for Wilm's tumor where it is overexpressed [42]. Recently, it was shown that *IGF2as* is exported in the cytoplasm and associated with polysomes [43]. So, it is not impossible that *IGF2as* is a protein coding transcript.

We have identified a non-coding transcript, antisense to *H19*, that we named 91H (**Figure 3**) [44]. This tran-

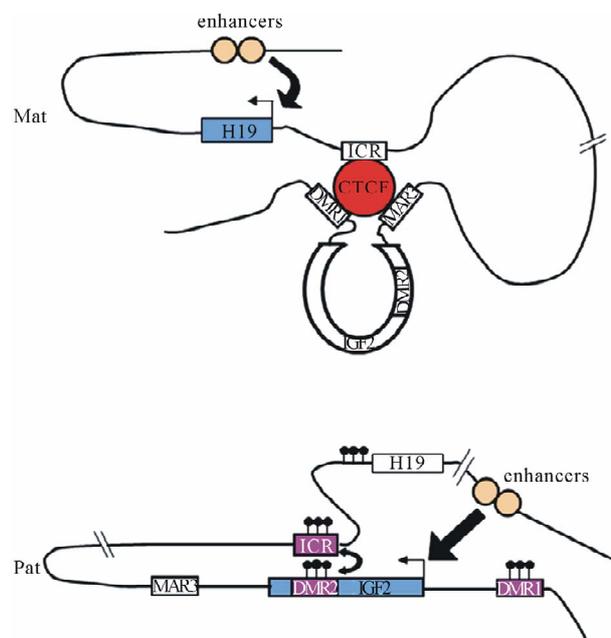


Figure 2. Chromatin loop structure at the *H19/IGF2* locus. Chromosome conformation capture revealed that the CTCF protein orchestrate chromatin structure. On the maternal allele, CTCF interact with ICR but also with DMR1 of *IGF2* and matrix attachment region (MAR3). These interactions create a loop around the *IGF2* gene. Then, enhancers can interact only with *H19* promoter and activate its expression. On the paternal allele, the methylated DMR2 of *IGF2* interact with the methylated ICR allowing thus moving of *IGF2* into the active chromatin domain.

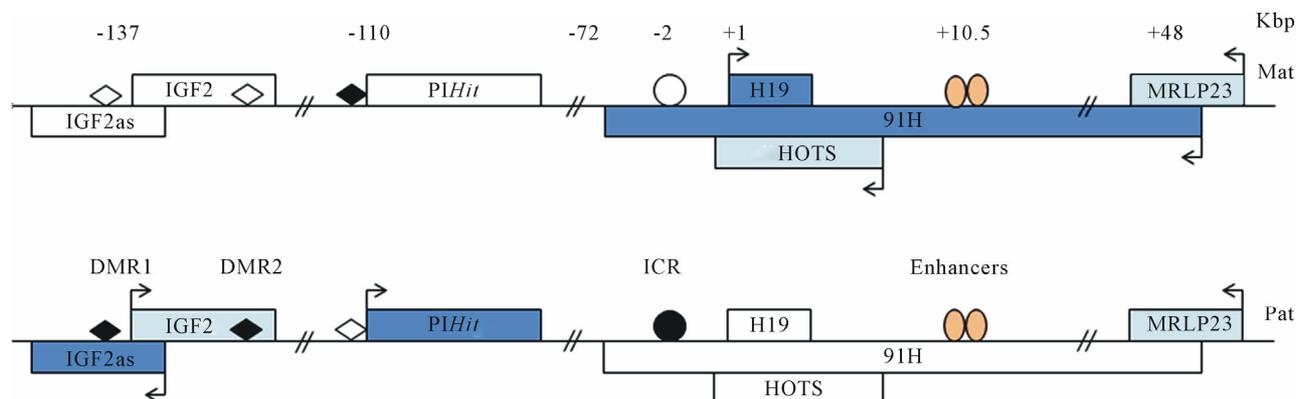


Figure 3. News transcripts at the *H19/IGF2* locus. Gene expressed are in blue rectangle with arrow whereas inactive gene are in white rectangle. Protein coding gene are represented by (□) and non-protein coding gene by (▭). Direction of arrows indicates direction of gene transcription. Relative positions are expressed in kilobase pairs relatively to the *H19* transcription start site. ICR is represented by circle and DMR by diamond. Methylation of ICR and DMR is in black. Enhancers are represented by orange oval. The first antisense transcript described is *IGF2as*. It is ncRNA overexpressed in Wilm's tumour. Its function remains unclear. Recently, news transcripts have been discovered at the *H19/IGF2* locus. On maternal allele, a long antisense transcript to *H19* was identified: it is *91H*. This ncRNA and its function are conserved in mice. Neither protein was identified, so it is supposed that *91H* acts as lncRNA. Recently, from the same allele, a protein coding gene imprinting has discovered: *HOTS*. This transcript is antisense to *H19*. It is possible that *HOTS* protein is coding by *91H* RNA. The last transcript discovered from *H19/IGF2* locus is *PIHit*. It is ncRNA expressed after birth from paternal allele.

script is a lncRNA of 120 kb expressed only in human from the maternal allele. It is known that lncRNAs can mediate epigenetic regulation transcription. Indeed, the lncRNA *Xist* induce X-chromosome inactivation in dosage compensation. So, we have studied effects of *91H* expression at the *H19/IGF2* locus. By invalidation of *91H* with si-RNA, a reduction of *IGF2* expression was observed. However, today, the molecular mechanism remains unclear. Recently, it was shown that *91H* RNA and its function are conserved among mammals, notably in mice [45]. By *91H* overexpression, they have shown that *91H* regulates positively *IGF2* translation from a novel promoter. More recently, a group identified antisense transcript of *H19*, expressed from the maternal allele, encoding for a protein named *HOTS* (*H19* opposite tumour suppressor) [46]. But it is not excluded that this protein is encoded by the *91H* transcript. Thus, today the function of the *91H* transcript remains unclear.

A new paternal transcript was identified in mice. This transcript, *PIHit* (Paternally-expressed *IGF2/H19* intergenic transcript), is coding by intergenic sequence, between *IGF2* and *H19*, and expressed, in mice, principally 8 days after birth (Figure 3) [47]. Then, its expression decreases rapidly during the third post-natal week. It is expressed at similar level to mRNA (*IGF2*), capped but no polyadenylated. Neither ORF was identified, so it is supposed that it is a lncRNA. Authors have identified transcription start site but not the 3' end, which is why it is a transcript of 5 to 6 kb. Neither function has been associated to *PIHit* RNA. By 3C, they observed two chromatin conformation of paternal allele. They supposed that there is a dynamic system permitting *IGF2* or

PIHit expression. However, it cannot exclude that there is chromatin conformation cell lines specific.

4. THE *H19* RNA AND ITS FUNCTION IN CELLS

4.1. The *H19* RNA

The *H19* gene was discovered in the mouse in 1984 and in the human in 1992 [48-50]. This gene is composed of five exons and encoded an mRNA of 2.3 kb. This RNA is transcribed by the RNA polymerase II, polyadenylated, capped and spliced with conserved secondary RNA structure. But, no conserved open reading frame was identified. Even if deletion and/or mutation produce a 26 kDa protein, no endogenous translation has so far been identified [51]. So, in 1990, Brannan *et al.* have proposed that *H19* RNA functions as a *riboregulateur* of which expression is developmentally regulated [52].

It is well established that a ncRNA can be precursor of microRNA. There are different biogenesis pathways of microRNA, but generally stem-loop structure RNA are recognized by protein like DGCR8, cleaved by Drosha and Dicer to generate the duplex miR-5p/miR-3p. Then, the duplex interacts with Argonaut protein family and is incorporated in the RISC complex. MicroRNA can also be generated in Drosha or Dicer-independent pathways [53]. Introns from the splicing or tRNA (tRNA-Ile for example) can be directly recognized by Dicer, cleaved by this enzyme and incorporated in the RISC. There is a microRNA (miR-451) cleaved by Drosha which is directly recognized by Ago and incorporated in the RISC.

In 2007, Cai and Cullen have demonstrated that *H19* is

precursor of microRNAs: miR-675-5p and miR-675-3p [19]. They are generated by the exon1 of the gene. Today, few targets of the miR-675 have identified. Due to its microRNA, it was shown that *H19* can regulate placental growth and cell cycle.

4.2. *H19/91H* Regulate *IGF* Associated Phenotypes

We and others groups have established that *H19* regulates *IGF2* ligand and receptor expression. Expression of *H19* and *IGF2* are regulated by enhancers located downstream of *H19*. Actions of enhancers are regulated by imprinting control region (ICR), located between *H19* and *IGF2*. Expression of *H19* and *IGF2* are allele-dependent. On the maternal allele, the CTCF protein interacts with ICR non-methylated, DMR1 of *IGF2* and the MAR3 domain [35,37,38]. This interaction creates a loop containing *IGF2* gene. Enhancers cannot active transcription of *IGF2* gene when chromatin is in this conformation. So, only *H19* is expressed from the maternal allele. On the paternal allele, ICR is methylated, so the protein CTCF is absent on this allele. ICR methylated interacts with *IGF2* DMR2 methylated too. This interaction allows action of enhancers on *IGF2* promoter and then *IGF2* expression [30]. *H19* and *IGF2* are in competition for enhancers. Furthermore, it was shown that deletion of *H19* and its flanking region affect expression of *IGF2*. So, *H19* and region flanking regulate *IGF2* expression in *cis*.

Moreover, *H19* is a RNA polyadenylated, spliced and exported in the cytosol. In cytosol, a group have shown that *H19* is associated to polysomes [54]. These polysomes have similar size to those associated to *IGF2* mRNA. Then, they have found an inverse co-regulation between *H19* expression and *IGF2* translation in cytosol. In Wilm's tumor, *H19*-negative cells show overexpression of *IGF2* 3 fold higher than control. Inversely, in *H19*-positive cells, *IGF2* expression protein was reduced. So, they hypothesized, that *H19* regulates translation of *IGF2* mRNA in *trans*. Moreover, it was shown a co-regulation between *H19* and *IGF2* transcription. In breast cancer cells, when *H19* is overexpressed, *IGF2* expression decreases severely [55,56]. *H19* regulates negatively transcription of *IGF2* in *trans*. So, it was supposed that *H19* acts as a *trans-riboregulateur*.

In mouse placental cells, the expression of miR-675, from *H19* gene, is regulated negatively by HuR protein [21]. They observed a relation between miR-675 expression and size of placenta. Indeed, when miR-675-3p is expressed (from E11.5 until term), a reduction size of placenta is observed. This reduction is due to a decrease of cells proliferation but not an increase of apoptosis. They established that miR-675-3p interacts with two seed on 3'UTR *IGF1r* and inhibits its translation. So,

H19 is a key regulator of *IGF* ligand and receptor expression (Figure 4).

Intriguingly, we have shown that the lncRNA *91H*, transcript antisense to *H19*, affects a little *H19* expression but regulates positively *IGF2* [44]. However, the mechanism remains unclear. We supposed that *91H* interacts with proteins that modulate expression of genes. More recently, a group have shown that *91H* overexpression, in mouse, upregulates *IGF2* expression [45]. They supposed that *91H* activate a novel promoter of *IGF2*.

4.3. Cell Cycle Regulation by *H19* Promotes Cancer

H19 is implicated in embryonic development. It is expressed in blastocyst stage of development and accumulated at high level in tissues of endodermal and mesodermal origins as well as ectodermal origin [57-60]. After birth, the gene is repressed in all tissues except skeletal muscle [61]. Misregulations of *H19* expression during development induce developmental syndrome like Silver-Russel syndrome or Beckwith-Wiedemann syndrome [62,63]. In adulthood, function of *H19* is controversial: it was supposed that *H19* act as tumor suppressor or oncogene. Nevertheless, several data show that *H19* act as oncogene in various cancer tissues: breast [61,64,65], uterus [66], bladder [67,68] and gastric [69]. Indeed, today, it was clearly established that *H19*, *per se* or through its microRNA, regulates different check-point of the cell cycle.

H19 mRNA generates two microRNAs: miR-675-5p and miR-675-3p [19]. The mir-675-5p is most studied but few targets have been identified. The first target identified is 3'UTR of Retinoblastoma (*RB*) mRNA in colon cancer cells [20]. Authors have shown a negative co-regulation between *H19* and *RB* in human colorectal

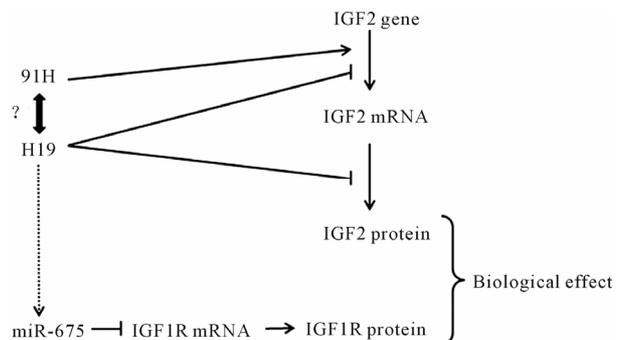


Figure 4. Regulation of *IGF* by *H19* and its antisense transcript *91H*. Arrows represent positive regulation (→) and vertical bar negative regulation (⊣). *H19* overexpression regulates negatively *IGF2* transcription and translation. The lncRNA *91H* regulates positively transcription of *H19* and *IGF2* but the mechanism is unclear. *H19* is precursor of microRNA: miR-675. MiR-675 inhibits translation of *IGF1r* mRNA. So, *H19* is a key regulator of *IGF* ligand and receptor.

tumour. They demonstrated a reduced expression of *RB* in tumour whereas *H19* and miR-675-5p are overexpressed (Figure 5). Owing to a reporter luciferase vector, they established the interaction and the negative effect of miR-675-5p on 3'UTR of *RB*. They have shown that miR-675-5p increases clonogenicity in soft agar of human colon cancer cells. So, in colon cancer cells lines, *H19* and miR-675-5p increase proliferation of cells.

In 1998, it was shown that the *H19* expression is regulated by p53 protein [70]. Indeed, the *H19* promoter contains consensus site to interaction with p53. A negative regulation of p53 on this promoter was observed. In parallel, in human breast tumour, *H19* is overexpressed in 70% of tumour independent of p53 expression [71]. However, it was shown that *H19* is located in stromal cells whereas p53 is located in epithelial cells.

Recently, a group studying gastric cancer shows that *H19* expression is increased in this disease [69]. They observed an increase cell proliferation and a reduction of apoptosis when *H19* is overexpressed. So, they studied the effect of *H19* overexpression on a protein inhibiting cell cycle proliferation and inducing apoptosis: the p53

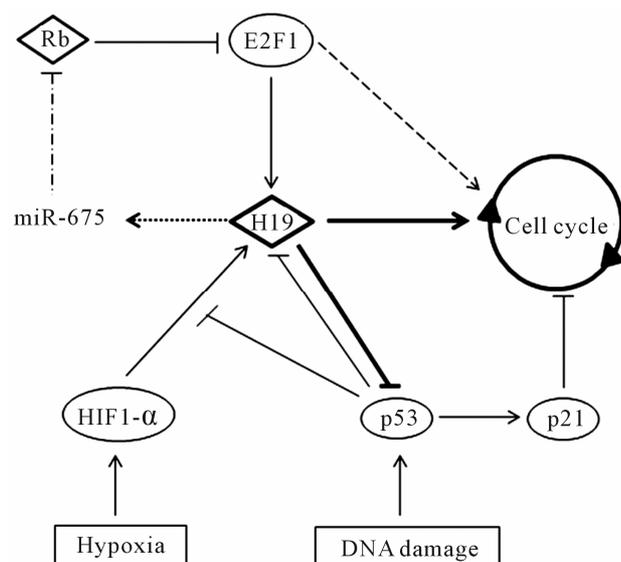


Figure 5. Regulation of cell cycle by *H19*. Proteins are represented by circle, RNA by diamond, cellular stress by rectangle and cell cycle by circle with arrow. Activation of gene expression is represented by arrow (→) and repression by vertical bar (—|). Mir-675 is processed from *H19* transcript and represses translation of an inhibitor cell cycle progression mRNA: *Rb* (*Retinoblastoma*). So, E2F1 protein is activated and promotes *H19* overexpression and expression of genes involved in cell cycle progression. *H19* overexpression facilitates G1/S transition. Hypoxia, by activating HIF1- α , induces *H19* expression in p53-dependent manner. DNA damage activates the p53 protein. P53 repress *H19* expression and inhibits cell cycle progression by activating p21. Recently, it was shown that *H19* overexpression inhibits p53 activity. So, *H19* is a key regulator of cell cycle progression.

protein. Thanks to a RNA-immunoprecipitation (RIP), they have shown that *H19* RNA can interact physically with the p53 protein. By a luciferase reporter system, they demonstrated that *H19* RNA regulates negatively the p53 protein may be by blocking this phosphorylation. So, the *H19* overexpression in gastric cancer cells contributes to tumorigenesis by regulating p53 activation.

In tumour, some cells are in hypoxic condition. So, a team has studied effect of hypoxia on *H19* expression. Upon hypoxia, they observed an increase rate of *H19* RNA [72,73]. So, they have verified that the activation is due to the activation of the HIF1- α pathway (pathway activated during hypoxia). Invalidation of HIF1- α by RNA interference induces a diminution of *H19* overexpression upon hypoxia. Furthermore, they have observed that *H19* is overexpressed only when p53 is mutated or absent. If, p53 is not mutated, they have observed a decrease *H19* expression. It has previously been reported that p53 inhibits action of HIF1- α by increasing its ubiquitination and degradation [74]. So, upon hypoxia, *H19* is overexpressed by activation of HIF1- α pathway and p53-dependent manner.

Surprisingly, in breast cancer cells (MCF-7), they have observed an overexpression of *H19* upon hypoxia although p53 is present. In this cell, the p53 protein is principally in the cytoplasm. So, to repress activation of transcription by HIF1- α , p53 must be in the nucleus. Taken these results together, we can hypothesize that, in MCF-7 cells, *H19* interacts with the p53 protein and inhibits its activation by sequestering p53 protein in the cytoplasm.

Furthermore, it was shown that *H19* facilitates cell cycle transition G1/S [75]. This check-point is regulated particularly by the E2F1 protein. The *H19* promoter contains two consensus sites for this protein. It was studied the potential role of E2F1 on the *H19* promoter. Using luciferase system, it was reported that E2F1 induced *H19* expression through these two sites. Moreover, the RB protein and E2F6 factor inhibit the activation of E2F1. So, E2F1 is negatively regulated by RB and E2F6. Recently, it was shown that *H19*, thanks to its microRNA, regulates negatively RB expression [20]. So, these studies have demonstrated a positive feedback loop between *H19* and E2F1 (Figure 5). Then, in breast cancer cells lines as BT20, T47D and MCF-7, E2F1 and *H19* are overexpressed. It was shown that *H19* overexpression conferred a growth advantage on cells. Indeed, an increase S-phase entry was observed when cells overexpress this gene [75]. So, *H19*, through a positive regulation by E2F1, active cell cycle progression and promotes growth of breast cancer cells.

To resume, it was shown that, in cancer cell lines, *H19* control p53 activity, reduced translation of the *RB* mRNA and promotes the G1/S cell cycle transition (Figure 5).

So, *H19* have a key role in the regulation of cell cycle and could be implicated in cancer progression.

6. CONCLUSION

Recently, the ENCODE project have proved that 90% of the genome is transcribed, but protein-coding genes represent only 2% of transcripts. So, the genome encode for ncRNAs which have an important place in function of cells. Today, small non-coding RNAs are most studied than lncRNAs. However, lncRNAs regulate the expression of 70% of genes. So general, it is accepted that lncRNAs regulate gene expression by interacting with PRC complex chromatin modifications, including PCR2. In this review, we show that lncRNA, notably *H19* RNA, may have several functions in cell without involving the complex. So, we show that lncRNAs can act *per se* and/or as precursor of microRNA. Furthermore, we show that lncRNAs can be involved in different cellular process. For example, the *H19* RNA and its microRNA regulate negatively expression of *IGF2* ligand and *IGF1* receptor; and control positively cell cycle progression. So, today, it is clear that lncRNAs have function as important as protein within cellular processes.

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