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Igf2-H19, an Imprinted Tandem Yin-Yang gene and its Emerging Role in Development, Proliferation of Pluripotent Stem Cells, Senescence and Cancerogenesis

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Among $3.0-3.5 \times 10^4$ genes in mammalian genome there are ~80 genes that are paternally imprinted and expressed from the maternal or paternal chromosome only. The most important among them is insulin-like growth factor-2 (*Igf2*)-*H19* locus that encodes genes that govern embryogenesis, fetal growth, behavioral development, the totipotent state of the zygote, and pluripotency of developmentally early stem cells [1-4].

This tandem gene regulates insulin like growth factor-1 (IGF-1) and insulin like growth factor-2 (IGF-2) signaling that regulates many vital aspects of cell biology. To support this while *Igf-2* encodes IGF-2, which is an autocrine/paracrine mitogen, transcription of *H19* gives rise to non-coding mRNA that is a precursor of several microRNAs (miRNAs) that negatively affect cell proliferation, including miR-675 that as recently demonstrated is involved in downregulation of insulinlike growth factor-1 receptor (IGF-1R) [5].

The expression of imprinted genes is regulated by the imposition of epigenetic marks by DNA methylation within differentially methylated regions (DMRs), which are CpG-rich *cis*-elements within their loci [1-4]. These epigenetic marks imposed on DMRs in the female germline act on the promoters of imprinted genes, which results in the heritable repression of the maternal chromosomes. In contrast, the imposition of epigenetic marks by methylation of the chromosomes in the male germline does not occur at the promoters but rather within the intergenic regions (e.g., on DMR between the *Igf2* and *H19* genes). Imprinted genes, in general, are highly expressed during embryogenesis, and many of them are subsequently downregulated after birth. Most of them are methylated on DMRs in maternally derived chromosomes and only few including *Igf2-H19*, are methylated on DMRs on paternally derived chromosomes [1-4].

Figure 1 upper panel shows a schematically simplified structure for this locus. The filled lollipops at the DMR regulatory region of the paternal chromosome depict methylation, and open lollipops on the maternal chromosome indicate lack of methylation. If the DMR is methylated, it cannot bind the regulatory DNA-binding zinc finger insulator protein, CTCF,

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which establishes a functional boundary between the *Igf2* and *H19* coding regions. The binding of CTCF has immediate consequences for expression of these loci. Since expression of both *Igf2* and *H19* is regulated from a 3' distal enhancer (shown as a green box), the presence of CTCF bound to the DMR at the maternal locus prevents transcription of *Igf2*, and in this situation, only *H19* is transcribed to RNA [1-4]. In contrast, the presence of a methylated DMR on the paternal chromosome prevents binding of CTCF, and in this situation, the 3' distal enhancer promotes transcription of mRNA from the *Igf2* locus. This ensures a proper balance in expression of both genes and the proper imprinting of DMR within this "Yin-Yang" locus, with methylation of the paternal chromosome and a lack of methylation on the maternal chromosome, regulates expression of both of these genes so that *Igf2* is transcribed only from the paternal (P) chromosome and *H19* only from the maternal (M) chromosome (Figure 1 upper panel).

According to the parent-offspring conflict theory, paternally expressed imprinted genes (e.g., *Igf2*) enhance embryo growth and maternally expressed genes (e.g., *H19*) inhibit cell proliferation and somehow negatively affect size of the offspring [1-4]. Accordingly, while during pregnancy, the father, through proper expression of paternally imprinted genes (e.g., by expression of *Igf2* and suppression of *H19*), contributes to body size and muscle mass of the developing fetus and wants the mother to devote as much of her resources as possible towards the growth of his offspring, the mother wants to conserve as much of her resources as possible towards future births (without compromising the health of the fetus she is currently carrying) by epigenetic modulation of genes by maternal imprinting marks (e.g., to achieve suppression of *Igf2* and expression of *H19*) [4].

On the other hand, erasure of genomic imprints including erasure of methylation at DMR of *Igf2-H19* locus (Figure 1 middle panel) is one of the mechanisms that plays an important role in regulating quiescence of pluripotent stem cells in adult organisms (e.g., primordial germ cells [PGCs] and very small embryonic-like stem cells [VSELs]) [6,7]. This mechanism of erasure of imprinting prevents these developmental early cells from uncontrolled proliferation and teratoma formation and may be involved in the regulation of life span [6,8]. Erasure of Imprinting at the *Igf2-H19* locus is one of the major factors preventing parthenogenetic development in mammals, and the biological importance of this locus is demonstrated by the additional steps necessary for creation of viable bimaternal mice derived from two female sets of chromosomes [9]. These mice are created by combining two haploid nuclei, one from non-growing and the other from fully growing oocytes, into a diploid "bimaternal zygote". Since female chromosomes have unmethylated DMRs for *Igf2-H19* (Figure 1 middle panel), the crucial step is appropriate genetic modulation of the proper expression of *Igf2* from one of the maternally derived sets of chromosomes to situation depicted in Figure 1 upper panel [9].

Modification of genomic imprinting also plays a crucial role in maintaining the pool of pluripotent stem cells residing in adult tissues. Recently, our group demonstrated that adult murine tissues harbor a population of pluripotent Oct4⁺ SSEA-1⁺ Sca-1⁺ Lin⁻ CD45⁻ cells [10]. We have also identified a corresponding population of cells that are Oct4⁺ SSEA-4⁺ CD133⁺ Lin⁻ CD45⁻ in humans as well. We hypothesize that these PSCs, which are called VSELs, are deposited in adult tissues, including during early embryogenesis, and serve as a backup for tissue-committed stem cells (TCSCs). Molecular analysis of VSELs has revealed that their quiescence in adult BM and premature depletion from the tissues is controlled by epigenetic changes to imprinted genes, including the *Igf2-H19* locus, which is erased in murine VSELs [7,8] (Figure 1 middle panel).

Based on these findings, we proposed a novel hypothesis that relates aging, longevity, and insulin-like growth factors signaling to the abundance and function of pluripotent VSELs

deposited in adult tissues. A decrease in the number of these cells should negatively affect pools of TCSCs in various organs and have an impact on tissue rejuvenation and life span [11]. In support of this expectation, we observed a significantly higher number of VSELs in long-living murine strains (e.g., Laron dwarfs and Ames dwarfs) whose longevity is explained by low levels of circulating IGF1 [12]. By contrast, the number of VSELs is reduced compared to normally aging littermates in mice with high levels of circulating IGF1 (e.g., growth hormoneoverexpressing transgenic mice) [12].

In contrast, hypermethylation of *Igf2-H19* locus on both chromosomes called in contrast to erasure of imprinting, loss of imprinting, results in *Igf2* overexpression (Figure 1 lower panel) and is observed as epigenetic change in several malignancies (e.g., rhabdomyosarcoma and nephroblastoma), where overexpressed IGF-2 acts as an autocrine growth factor for tumor cells. The best example, as mentioned above, is Beckwith-Wiedemann syndrome, which is associated with the development of several pediatric sarcomas [13].

In conclusion, Evidence has accumulated that the imprinted *Igf2-H19* tandem gene plays a pleiotropic role in several biological processes. Expression of this locus is tightly regulated by genomic imprinting, which ensures the balanced expression of both genes from either paternal or maternal chromosomes. Modification of expression at the *Igf2-H19* locus may have an important role in inhibiting aging processes and preventing cancerogenesis. Furthermore, as we envision, proper methylation of the DMR at this locus, which is erased in VSELs [12,13], will be crucial for development of *ex vivo* strategies for expansion of VSELs for the purposes of regenerative medicine [14].

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Figure 1. Changes in methylation state of DMRs and their impact on *Igf2* and *H19* expression
Upper panel. *Igf2* and *H19* coding regions are separated by a differentially methylated region (DMR) that is methylated (as shown by filled lollipops) on the paternal chromosome (P) and unmethylated (open lollipops) on the maternal (M) chromosome. Expression of both genes is regulated by a 3' distal enhancer depicted in green. Methylation of the DMR on the paternal chromosome (P) prevents binding of CTCF insulator protein and allows activation of the *Igf2* promoter by the distal enhancer and transcription of *Igf2* mRNA from the paternal chromosome (M) (red arrow). In contrast, since the DMR is unmethylated on the maternal chromosome (M), it binds CTCF, and this prevents activation of the *Igf2* promoter by the distal enhancer. As a result, only *H19* mRNA is transcribed from the maternal chromosome (P) (red arrow). Normal somatic imprint observed in all somatic cells, which results, as described in Figure 1, in properly balanced expression of *Igf2* from the paternal chromosome and *H19* from the maternal chromosome (red arrows). **Middle panel** – Erasure of imprinting at the *Igf2-H19* locus as seen in primordial germ cells (PGCs) and pluripotent stem cells residing post-developmentally in adult tissues (VSELs). DMRs on both the paternal (P) and maternal (M) chromosomes are engaged by the CTCF insulator protein, and thus only *H19* mRNA is transcribed (red arrows), contributing to the quiescent state of cells (lacking autocrine IGF2). **Lower panel** – Loss of imprinting at the *Igf2-H19* locus as seen in tumor cells from several types of cancer (e.g., rhabdomyosarcoma, neuroblastoma, and gastrointestinal tumors). Since both DMRs are methylated, the insulator protein CTCF cannot bind to the DNA and the distal enhancer stimulates transcription of mRNA for IGF2 from both chromosomes (red arrows). Cells that have this epigenetic change are under autocrine IGF2 stimulation.