REVIEW

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Igf2-H19, an imprinted tandem gene, is an important regulator of embryonic development, a guardian of proliferation of adult pluripotent stem cells, a regulator of longevity, and a 'passkey' to cancerogenesis

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Abstract: The insulin-like growth factor-2 (*Igf2*)-*H19* locus encodes important paternally imprinted genes that govern normal embryonic development. While *Igf-2* encodes IGF2, 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. The proper imprinting of a differentially methylated region (DMR) within this 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 chromosome and *H19* only from the maternal chromosome. There is growing evidence that this 'Yin-Yang' locus regulates embryonic development. Furthermore, recent evidence indicates that erasure of imprinting (hypomethylation) of the *Igf2-H19* locus on both chromosomes, which leads to downregulation of *Igf2* and upregulation of *H19* expression, plays an important role in regulating quiescence of pluripotent stem cells in adult organisms, and may be involved in the regulation of lifespan. In contrast, hypermethylation of this locus on both chromosomes (loss of imprinting) results in *Igf2* overexpression and is observed in several malignancies. In this review, we will discuss the biological consequences of changes in *Igf2-H19* expression. (*Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 2, 171–179*)

Key words: imprinting, Igf2, H19, VSELs, longevity, cancerogenesis

Introduction

Mammalian development requires proper gene dosage, which is enabled by the imprinting of genes so that a single parental allele is expressed. In other words, genomic imprinting is an epigenetic program that ensures the parent-of-origin-specific monoallelic transcription of imprinted genes and results in intracellular expression of imprinted genes from only one of the two paternal chromosomes, derived either from the mother or the father [1–5].

Overall, there are ~100 paternally imprinted genes in the mammalian genome (expressed from the maternal or paternal chromosome only) that play an important role in embryonal development. Imprinted genes play a crucial role in embryogenesis, fetal growth, behavioral development, the totipotential state of the zygote, and pluripotency of developmentally early stem cells [4]. On the other hand, erasure of genomic imprints is one of the mechanisms that prevent some of the pluripotent stem cells (e.g. primordial germ cells [PGCs] and very small embryon-

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ic-like stem cells [VSELs]) from uncontrolled proliferation and teratoma formation [6–13].

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 (i.e. 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 imprinted genes are methylated on maternally derived chromosomes, and only four: *Igf2-H19*, *RasGrf1*, *Dlk1/Dio3,* and *Zdbf2,* are methylated on paternally derived chromosomes [1–4]. In this review, we will focus on the biological consequences of imprinting within the DMR for *Igf2-H19*.

The *Igf2-H19* tandem gene is paternally imprinted in humans as well as in mice and, as mentioned above, the regulatory DMR for this gene is methylated on the paternal, and erased on the maternal, chromosome [1–4]. Figure 1 shows a schematically simplified structure for this locus. The filled lollypops at the DMR regulatory region of the paternal chromosome depict methylation, and open lollypops 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, which establishes a functional boundary between the *Igf2* and *H19* coding regions. The binding of CTCF has immediate consequences for the expression of these loci. Since the 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. 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 the expression of both genes (Figure 2, upper panel).

As discussed in this review, the methylation of DMRs within the *Igf2-H19* locus is erased in pluripotent stem cells, such as PGCs [6–11] and VSELs [12, 13] (Figure 2, middle panel). This epigenetic modification of *Igf2-H19* is one of the mechanisms responsible for keeping these cells quiescent (low expression of *Igf2* and overexpression of *H19*) and prevents

them from uncontrolled proliferation and teratoma formation. In contrast, hypermethylation of DMRs within *Igf2-H19* on both maternally and paternally derived chromosomes, which is called 'loss of imprinting', is observed in several malignancies and results in high expression of *Igf2* (Figure 2, lower panel). Loss of imprinting at this locus, as will be discussed later, could also result from perturbation of other mechanisms that regulate the proper *Igf2:H19* expression ratio in these cells (e.g.downregulation of CTCF expression) [14].

In this review, we will discuss the three possibilities (normal somatic imprinting, erasure of imprinting, and loss of imprinting) as epigenetic changes in methylation of DMRs for the *Igf2-H19* 'master locus' that affect several biological processes, such as proper embryogenesis, proliferation of pluripotent stem cells, senescence, and tumorogenesis.

Biological role of proper somatic imprinting of the Igf2-H19 tandem gene

The tandem *Igf2-H19* locus is located on chromosome 11p15 in humans and chromosome 7 in mice. While the *Igf2* locus encodes insulin-like growth factor-2 (IGF2), which is a growth-promoting peptide hormone highly expressed during embryogenesis, the *H19* locus encodes a non-coding RNA (ncRNA) that gives rise to several microRNAs (miRNAs) that have opposite effects on cell proliferation [15]. The dual Yin-Yang function of this master locus is relevant to several biological functions, including normal fetal development, and the properly balanced expression of Igf2 and H19 is crucial for proper embryogenesis (Figure 2 upper panel). 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 the creation of viable bimaternal mice derived from two female sets of chromosomes [1–4]. 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), the crucial step is appropriate genetic modulation of the proper expression of *Igf2* from one of the maternally derived sets of chromosomes [6–13].

The role of imprinted genes in development, including the master tandem gene *Igf2-H19,* has been explained by the parent–offspring conflict hypothesis. While during pregnancy, the father, through proper expression of paternally imprinted genes (i.e. by expression of *Igf2* and suppression of *H19*), contrib-
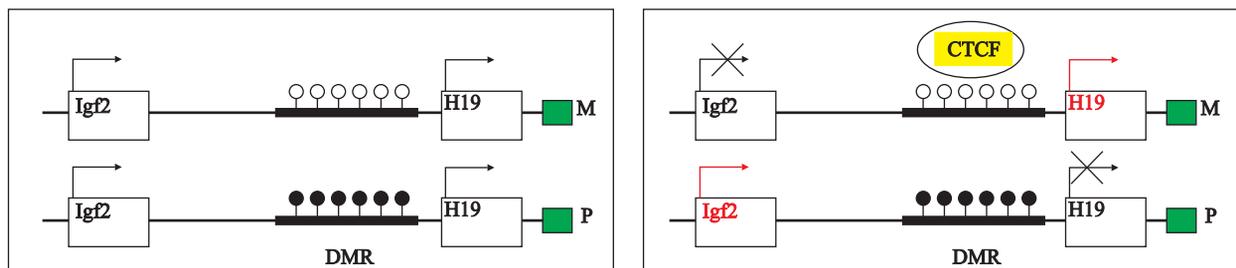
Figure 1. Regulation of expression of the *Igf2-H19* tandem gene. Left panel — *Igf2* and *H19* coding regions are separated by a differentially methylated region (DMR) that is methylated (as shown by filled lollypops) on the paternal chromosome and unmethylated (open lollypops) on the maternal chromosome. Expression of both genes is regulated by a 3' distal enhancer, depicted in green. Right panel — methylation of the DMR on the paternal chromosome 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 (red arrow). In contrast, since the DMR is unmethylated on the maternal chromosome, 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 (red arrow)
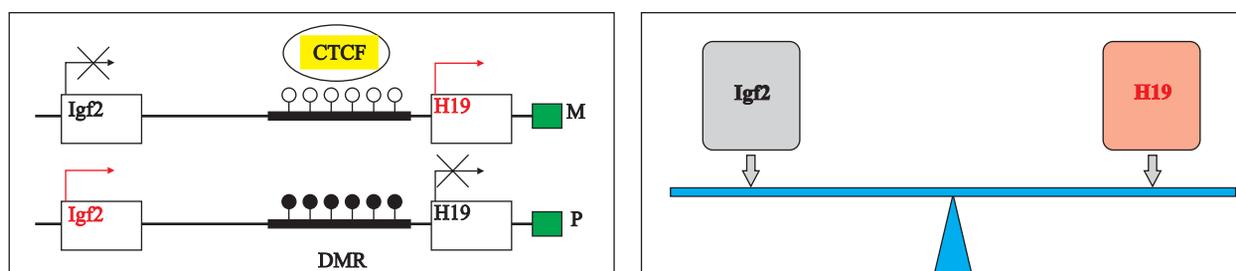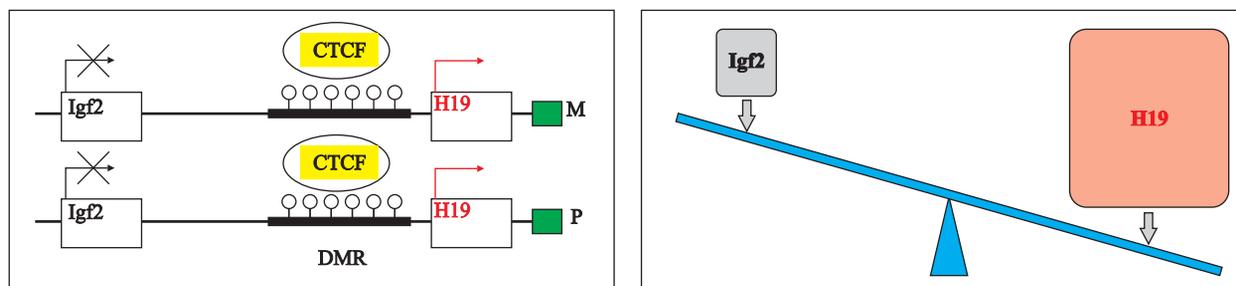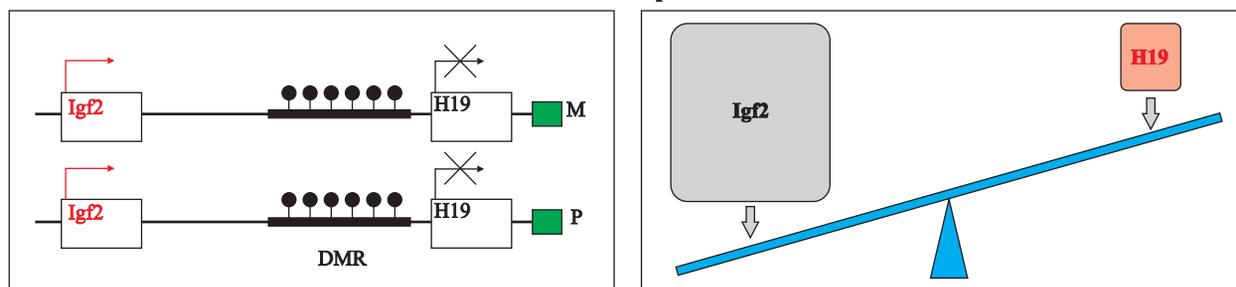

Figure 2. Changes in methylation state of DMRs and their impact on *Igf2* and *H19* expression. Upper panel —— normal somatic imprint observed in all somatic cells, which results, as described in Figure 1, in properly balanced expression of *Igf2* from the paternal chromosome and *H19* from the maternal 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 and maternal 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, nephroblastoma, 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

utes 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 carrying) by epigenetic modulation of genes by maternal imprinting marks (i.e. to achieve suppression of *Igf2* and expression of *H19*) [4].

It is well known that IGF2 is secreted from cells in an autocrine or paracrine manner and exerts its biological effects after binding to the insulin-like growth factor-1 (IGF1) receptor (IGF-1R). On the other hand, IGF2 may also bind to non-signaling insulin-like growth factor-2 receptor (IGF-2R), which is a mannose 6-phosphate receptor that acts as a decoy receptor for IGF2 and prevents its signaling through IGF-1R [17]. Overall, the IGF2 level is high in humans during embryogenesis and in postnatal life may be produced and secreted by some tumors, causing their proliferation and hypoglycemia as part of the paraneoplastic syndrome. The loss of imprinting at the *Igf2-H19* locus (Figure 1, middle panel) leads to overexpression of *Igf2,* which is observed in Beckwith––Wiedemann syndrome and is associated with organomegaly of newborns and a high incidence of several pediatric sarcomas (e.g. rhabdomyosarcoma and nephroblastoma) [18, 19].

In contrast, *H19,* as mentioned above, is a long, 2.3-kb, non-coding RNA (ncRNA) transcribed from the maternally inherited allele only; it is highly expressed prenatally and downregulated postnatally. It is well known that *H19* ncRNA, which is evolutionarily conserved at the nucleotide level in humans and rodents and not translated to protein, is processed into small miRNAs [15]. This is supported by the finding that *in situ* hybridization of the H19 ncRNA revealed that it is detectable in cytoplasmic ribonucleoprotein particles, which suggests that *H19*-derived miRNAs are involved in ribosomal function and translation. However, the loss of *H19* is not lethal in mice, http://en.wikipedia.org/wiki/H19_(gene) and such animals display an overgrowth phenotype similar to babies with Beckwith–Wiedemann syndrome, while the overexpression of *H19* is a dominant lethal mutation; mouse embryos overexpressing *H19* die after embryonic day 14.

The close coupling of *Igf2* and *H19* expression is explained by the fact that these two genes share the same 3' gene enhancer (Figure 1). It has been reported that deletion of this 3' enhancer results in downregulation of both *Igf2* and *H19* expression. Furthermore, there are some indications that the 3' enhancer has a more robust effect on the expression of *H19*

than *Igf2*. This could be explained by the facts that: 1) *H19* has a stronger promoter than *Igf2* [4, 14]; and 2) that the *H19* gene is physically closer to the 3' enhancer than *Igf2* (Figures 1 and 2).

The expression of *Igf2-H19* is regulated from the 3' enhancer by the transcriptional zinc finger DNA-binding protein repressor, CTCF (also known as CCCTC-DNA sequence binding factor). The human genome contains nearly 15,000 CTCF insulator sites, suggesting a widespread role of CTCF in gene regulation [5, 14, 16]. What is of importance for this review is that this DNA-binding regulatory protein is involved in insulator activity for *Igf2* expression by binding to the DMR at the *Igf2-H19* locus (Figure 1). Binding to the methylation-free DMR by CTCF blocks the interaction between the 3' distal enhancer and the promoter of *Igf2*, while not affecting *H19* expression. Overall, four CTCF binding sites have been identified in the murine *Igf2-H19* DMR and seven in the human counterpart. Interestingly, the human *Igf2-H19* DMR is not able to function when introduced as a transgene into the murine genome, which suggests differences in the regulation of this DMR between species [14].

In conclusion, proper imprinting of the *Igf2-H19* locus is required for the balanced expression of both genes in somatic cells and in normal embryonic development.

Biological consequences of erasure of imprinting at the DMR for the Igf2-H19 tandem gene in primordial germ cells (PGCs)

The erasure of methylation in the DMR for *Igf2-H19* (Figure 2, middle panel) leads to downregulation of growth-promoting *Igf2* from both paternal and maternal chromosomes and overexpression of proliferation-limiting *H19*. Therefore, cells that undergo such epigenetic modification tend to remain in a quiescent state, and this occurs early in the development of PGCs [6–11]. Accordingly, PGCs, while they migrate during embryogenesis through the embryo proper into the genital ridges, erase several maternally and paternally imprinted loci, including the *Igf2-H19* locus. This mechanism of erasure of imprinted marks has several important consequences. First, it prevents these cells from uncontrolled proliferation and the potential risk of teratoma formation. Second, it creates in mammals a barrier against parthenogenetic development [4].

Furthermore, after the erasure of imprinting, PGCs are not able to proliferate *in vitro* and do not complement blastocyst development, while nuclei harvested from PGCs are not capable of performing as DNA donors in therapeutic cloning. However, all

these limitations in the pluripotency of PGCs are reversed when their imprinting is re-established during *ex vivo* generation from PGCs of embryonic germ cells (EGCs) [6–11].

During normal development, proper imprinting in germ line cells is established in later stages when PGCs, after colonization of the genital ridges, develop into gametes — oocytes or sperm. Proper imprinting is established in female germ line (paternal imprinting) and male germ line (maternal imprinting) cells after a first meiotic division when the chromosomes are reduced to haploid number. Thus, when haploid gametes fuse during fertilization, the chromosomes in the diploid zygote have proper imprinting, including at the *Igf2-H19* loci.

As mentioned above, the importance of proper imprinting of *Igf2-H19* during embryogenesis has also been demonstrated during generation of viable bimaternal mice [20]. The bimaternal zygote is generated from two female sets of chromosomes without participation of a paternal genome by combining two haploid nuclei from non-growing oocytes — one derived from a newborn pup and another from a fully grown oocyte. Since female chromosomes have unmethylated DMRs for *Igf2-H19*, the proper expression of *Igf2* and *H19* in this mouse was achieved by appropriate genetic modification of the *Igf2-H19* locus on one set of female chromosomes to ensure that this locus expresses *Igf2* [20].

Biological consequences of erasure of imprinting in the DMR of the Igf2-H19 tandem gene in very small embryonic-like stem cells (VSELs)

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 [21, 22]. We have also identified a corresponding population of cells that are Oct-4+SSEA-4+CD133+Lin−CD45− in humans as well [23, 24]. 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 [12, 13] (Figure 2, middle panel).

Murine VSELs, in addition to erasure at the *Igf2-H19* locus, also modify expression of other imprinted genes. For example, we observed that murine BM-sorted VSELs erase the paternally methylated imprints (e.g. DMRs at the *Igf2-H19* and *RasGrf1* loci, Figure 3), while they hypermethylate the maternally methylated imprints (e.g. DMRs at *Igf2R*). The epigenetic modification of imprinted loci (including *Igf2-H19*) prevents VSELs from uncontrolled proliferation and, as in the case of PGCs, prevents them from teratoma formation. The epigenetic modification of imprinted genes also explains why VSELs, despite expressing several markers of pluripotency (e.g. open chromatin structure at the promoters for Oct-4 and Nanog), the presence of bivalent domains at developmentally important homeobox-domain containing genes, the reactivation of the X chromosome in female VSELs, and *in vitro* differentiation into cells from all three germ layers, do not complement blastocyst development after injection into the pre-implantation blastocyst [12, 13].

There are also other important implications for erasure of paternally imprinted genes in VSELs. Namely, the erasure at the paternally imprinted *Igf2-H19* and *RasGrf1* loci leads to impair insulin/insulin-like growth factor signaling (IIS) in these cells [25–27]. This has a potential impact on the role of VSELs in tissue rejuvenation and aging as well as on the involvement of these cells in tumorogenesis.

Erasure of imprinting at the Igf2-H19 locus in VSELs and its implications for tissue rejuvenataion and aging

The abovementioned changes in expression of imprinted genes in VSELs lead to perturbation of IIS by downregulation of IGF2, which is an autocrine factor involved in proliferation of VSELs, and downregulation of RasGRF1, which is a GTP-exchange factor (GEF) crucial for signaling from IGF-IR and the insulin receptor (InsR, Figure 3). In addition, in VSELs we observed hypermethylation of DMRs on the maternal chromosome encoding IGF-2R [12, 13], which, as mentioned above, serves as a decoy receptor that prevents IGF-2 from binding to IGF-IR [25]. Overall, this epigenetic reprogramming of genomic imprinting negatively affects IIS signaling, maintains the quiescent state of VSELs and thus protects them from premature depletion from the tissues, and prevents their involvement in tumor formation.

Based on these findings, we propose a novel hypothesis that relates aging, longevity, and IIS 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
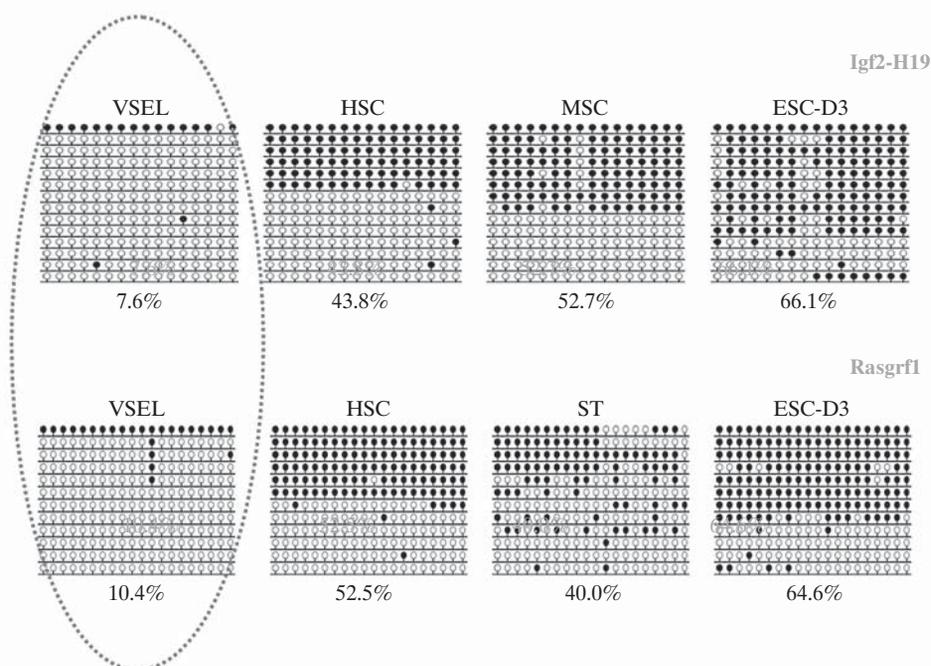
Figure 3. Erasure of genomic imprinting for paternally methylated imprinted genes in murine VSELs, HSCs, MSCs, and the ES-D3 cell line. Bisulfite sequencing profiles of DNA methylation of DMRs for *Igf2-H19* (upper panel) and *RasGrf1* (lower panel) loci in VSELs, hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and the murine embryonic stem cell line ES-D3. The percentage of methylated CpG sites was demonstrated by employing bisulfite modification and sequencing

lifespan [25–28]. 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 [29–32]. In contrast, the number of VSELs is reduced compared to normally aging littermates in mice with high levels of circulating IGF1 (e.g. growth hormone-overexpressing transgenic mice) [32].

Attempting to summarize all of this, the influence of IIS on the pool of VSELs residing in adult tissues seems to be twofold (Figure 4). In the first scenario, IGF2 is an autocrine factor for these cells, explaining why both downregulation of expression of *Igf2* and *RasGrf1* together with elevated expression of *H19* and upregulation of *IGF-2R,* which is a decoy receptor for IGF2, keeps these cells quiescent. However, we observed that with increasing age, the methylation pattern of DMRs at paternally imprinted genes in VSELs reverses and *Igf2-H19,* as well as *RasGrf1* loci, become gradually methylated, and thus VSELs become more sensitive to IIS [26–28]. This phenomenon may contribute to their age-related depletion over time.

On the other hand (second scenario), VSELs express functional IGF-1R and InsR and are susceptible to exogenous circulating IGF1 and insulin (Ins). Therefore, a chronic increase in caloric uptake that elevates circulating levels of these hormones may con-

tribute over time to the depletion of these cells from adult tissues, affect the generation of VSEL-derived TCSCs, and thus negatively affect lifespan. In support of this scenario, we demonstrated that mice that have high levels of circulating blood plasma IGF1 show accelerated depletion of VSELs and have a shorter lifespan than age-matched littermates.

Erasure of imprinting at the Igf2-H19 locus in VSELs and its potential role in cancerogenesis

The changes in imprinting of the *Igf2-H19* locus may be involved in tumor formation. A growing body of evidence suggests that cancer originates in the stem/ /progenitor cell compartment. This stem-cell-origin--of-cancer hypothesis is based on the assumption that self-renewing stem cells residing in organs and tissues, and not mature differentiated somatic cells such as those lining the bronchial or stomach mucosa, may accumulate mutations over a lifetime [33, 34]. These mutations are subsequently maintained in stem cell compartments, and self-renewing stem cells may be subjected to additional mutations and epigenetic changes so that the genome is destabilized and uncontrolled neoplastic proliferation is initiated.

Interestingly, during the 19th and early 20th centuries, several investigators proposed that cancer de-
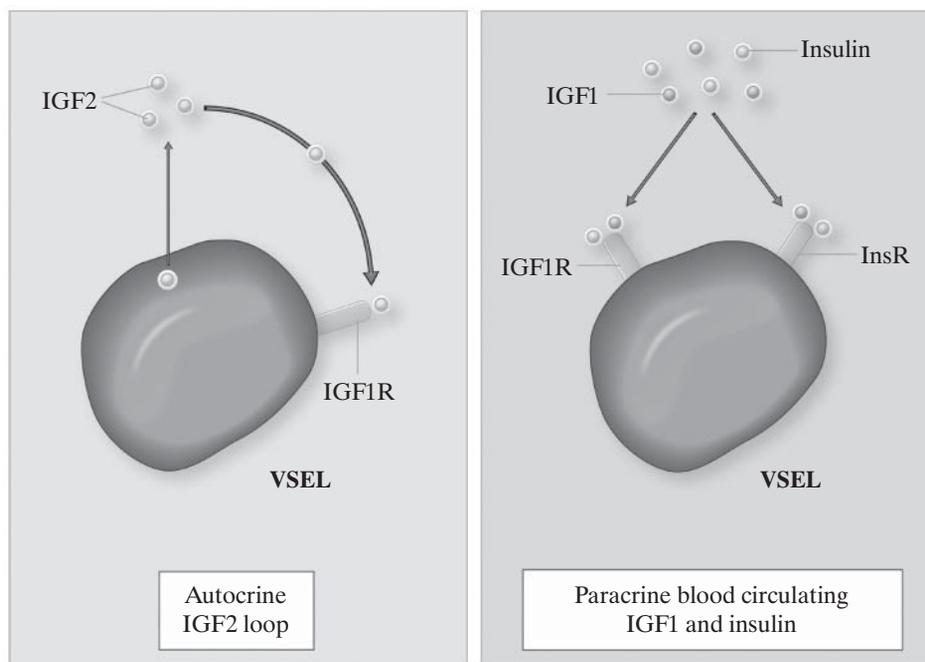
Figure 4. Two scenarios for how insulin/insulin-like growth factor signaling (IIS) may affect the pool of VSELs. VSELs are deposited in adult tissues as a backup population for tissue committed stem cells. Left panel — increase in autocrine--secreted IGF2, due to gradual loss of imprinting at the *Igf2-H19* locus as observed during aging, stimulates VSELs via IGF-1R. This leads to an age-mediated VSEL depletion, and as a consequence a decrease in VSEL-generated tissue--committed stem cells (TCSCs), and impairs tissue organ rejuvenation. Right panel — VSELs deposited in adult tissues may, over time, become depleted more quickly by chronic elevated circulating levels of IGF1 and insulin that engage IGF1R and InsR expressed by these cells. This may contribute to accelerated aging observed in situations with high circulating IGF1 and insulin level (e.g. high calorie uptake). On other hand, this mechanism of chronic exposure to IIS may also lead to malignant transformation of VSELs after prolonged stimulation by IGF1 and insulin. For the sake of simplicity, two other proteins encoded by imprinted genes that affect IIS are not shown — paternally imprinted *RasGrf1,* which due to erasure of imprinting is not expressed in VSELs, and maternally imprinted *IGF-2R,* which due to hypermethylation of the DMR locus on the maternal chromosome, is highly expressed in these cells. While RasGRF1 is an important component of IIS from activated IGF-1R and InsR, IGF-2R serves as a decoy receptor for IGF2. Therefore, changes in expression of these genes in VSELs further decrease the responsiveness of these cells to IIS

velops in populations of cells that are left in a dormant state in developing organs during embryogenesis [34]. This 'embryonic rest' hypothesis of cancer origin suggested that adult tissues contain embryonic remnants that normally lie dormant, but can be activated to become cancerous. Based on the presence of VSELs in adult tissues, we hypothesize that VSELs could be the missing link that reconciles the embryonic rest hypothesis of cancer origin with current theories envisioning cancer as a stem cell disorder.

In support of this perspective, it is well known that obesity and high caloric uptake are risk factors for cancer development. Experimental animals with high levels of circulating IGF1 are not only short-lived but also have a high incidence of cancer [35]. On the other hand, long-living animals with low levels of circulating IGF2 have a much lower incidence of tumor growth [32]. This correlates with human Laron dwarfs that have a very low level of circulating IGF1 and at the same time are prone to cancer development [29,

32]. Based on these observations, we envision that chronic stimulation of VSELs by IIS may activate these cells in an uncontrolled way and promote their malignant growth. Therefore, it is likely that some human tumors originate in VSELs, and that IIS may play an important initiating role. Again, we envision here two possible mechanisms. Firstly VSELs exposed to a constant high circulating IGF1 level could transform into neoplastic cells; and secondly, as discussed below, they could transform due to a loss of imprinting at the *Igf2-H19* locus that will expose them to autocrine IGF2.

Consequences for tumorogenisis of the loss of imprinting at the DMR for the Igf2-H19 tandem gene

There is growing evidence that the autocrine IGF2––IGF-1R loop, due to loss of imprinting at the *Igf2-H19* locus (Figure 2, lower panel), may be involved

in the growth of several types of tumors. The best example, as mentioned above, is Beckwith–Wiedemann syndrome, which is associated with the development of several pediatric sarcomas. The loss of imprinting at the *Igf2-H19* locus is a very well-known phenomenon observed both in Beckwith–Wiedemann syndrome-related and spontaneous human rhabdomyosarcomas and nephroblastomas. Loss of imprinting at this locus has also been described in gastrointestinal tumors, both in humans and in mice [36–39].

Furthermore, as mentioned above, one of the features of aging VSELs is gradual hypermethylation of DMRs for *Igf2-H19* locus on the maternal chromosome that due to this 'age-related' loss of imprint leads to overexpression of IGF2 [26–28]. This may correspond to a higher incidence of cancer in advanced age. The deregulation of the *Igf2-H19* locus observed in several tumors raises the question of the cell of origin in which this genetic defect originates. As discussed above, one possibility is that this event occurs in VSELs [33, 34]. This hypothesis, however, requires further studies.

Conclusions

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 and the CTCF protein, which is involved in the balanced expression of both genes from either paternal or maternal chromosomes. In addition, an interesting mechanism has been described in which elevated levels of IGF2 in senescent human epithelial cells are the result of a reduction in CTCF expression, which controls the *Igf2-H19* locus. As reported, an increase in the intracellular CTCF level, leading to lower occupancy of DMRs by CTCF within *Igf-H19* loci, resulted in a 10-fold increase in intracellular *Igf2* expression [14].

Furthermore, it is likely that, in addition to CTCF, other proteins are also involved in the regulation of this locus and that they still await identification. 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 the development of *ex vivo* strategies for the expansion of VSELs for the purposes of regenerative medicine [40].

Acknowledgments

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