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Survey

IGF2: Epigenetic regulation and role in development and disease

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Abstract

Insulin-like growth factor II (IGF2) is perhaps the most intricately regulated of all growth factors characterized to date. Its gene is imprinted – only one allele is active, depending on parental origin – and this pattern of expression is maintained epigenetically in almost all tissues. IGF2 activity is further controlled through differential expression of receptors and IGF-binding proteins (IGFBPs) that determine protein availability. This complex and multifaceted regulation emphasizes the importance of accurate IGF2 expression and activity. This review will examine the regulation of the *IGF2* gene and what it has revealed about the phenomenon of imprinting, which is frequently disrupted in cancer. IGF2 protein function will be discussed, along with diseases that involve IGF2 overexpression. Roles for IGF2 in sonic hedgehog (Shh) signaling and angiogenesis will also be explored.

Keywords: Insulin-like growth factor; Imprinting; Angiogenesis; Vascular endothelial growth factor; Sonic hedgehog

Contents

1. Introduction

In the early 1900s, the innovative surgeon Alexis Carrel experimented with maintaining tissues and whole organs *in vitro*, hoping to advance techniques in organ transplantation. Carrel observed that certain tissue extracts could induce cell proliferation, and he published his findings with this disclaimer:

"Possibly the finding of the activating power of tissue extracts will have no immediate practical application. Nevertheless, it may be indirectly useful by leading to the discovery of some of the factors determining the growth of tissues and of the unknown laws of cell dynamics . . . [1]."

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Table 1
A brief history of the insulin-like growth factors (IGFs)

1912	Albert Schaefer coins the term "insulin" for a substance in blood that controls glucose metabolism [2]
1913	The landmark article "Artificial activation of the growth *in vitro* of connective tissues" is published [1]
1957	Serum is found to contain "sulfation factor activity" (SFA), which mediates the effect of growth hormone (GH) on sulfate uptake by cartilage [3]
1963	The insulin-like factor in human serum, that is not neutralized by anti-insulin antibodies, is given the term "non-suppressible insulin-like activity" (NSILA) [4]
1972	Because SFA and NSILA have similar (if not identical) activities, the term "somatomedin" is proposed to denote the ability to promote somatic growth [5]
1973	"Multiplication-stimulating activity" (MSA), which induces proliferation of chicken embryo fibroblasts, is found in rat liver cell-conditioned media [6]
1976	NSILA is sequenced and found to be two distinct proteins, similar to human and tuna fish insulin. They are named IGF1 and IGF2 [7]
1981	MSA is purified, sequenced, and found to differ from human IGF2 by only five amino acids. Thus, MSA is designated as rat IGF2 [8]
1987	The IGF nomenclature is adopted to denote SFA, NSILA, somatomedin, and MSA [9]

Carrel was mistaken that this finding would have no practical application—rather, it pioneered the discipline of tissue culture and the widespread use of serum to support *in vitro* cell growth. He was right, however, that this "activating power" would eventually lead to the discovery of growth factors, many of which were isolated and characterized in the decades that followed. Two of these factors, which were structurally similar to insulin, had many effects on cell growth and differentiation. In 1987, after 30 years of confusing nomenclature, these proteins were designated as insulin-like growth factor I (IGF1) and insulin-like growth factor II (IGF2) (Table 1).

The IGFs regulate cell growth and differentiation in many species. The anabolic functions of growth hormone are largely mediated by IGF1, which designates IGF1 as a major determinant of somatic growth [10]. Rare mutations in the human *IGF1* gene lead to severe growth inhibition and mental retardation [11]. *Igf1*-null mice are born at 60% of normal birth weight, and the few that survive to adulthood are less than one-third the size of normal mice [12,13]. On the other hand, IGF2 is virtually dispensable for post-natal development in mice, since *Igf2* expression is almost entirely limited to the embryo in rodents [14]. At birth, *Igf2*-null mice are also growth-impaired but are otherwise normal, and subsequent growth proceeds at normal rates [13].

These studies support a somewhat redundant role for IGF2; furthermore, its designation as the "second" IGF seems to have relegated it to a lesser role than IGF1. However, IGF2 is the predominant IGF in adult humans (reviewed in Ref. [15]), and inappropriate IGF2 expression is implicated in a growing number of diseases (reviewed in Ref. [16]). The importance of IGF2 is highlighted by its complex and multifaceted regulation. The gene that codes for IGF2 is imprinted such that only one allele is expressed, depending on parental origin [14]. Besides the intriguing mechanisms that surround its imprinted expression, IGF2 is further modulated by a concert of differentially expressed proteins and receptors that determine IGF availability (reviewed in Ref. [17]). This review will examine the complex epigenetic regulation of the *IGF2* gene and provide a broad introduction to IGF2 signaling. The ability of IGF2 to stimulate cell proliferation and differentiation will be reviewed, which will lead to a discussion on its involvement in various cancers and other diseases. The angiogenic functions of IGF2 will be addressed, and conclude with a proposal that IGF2 is a key mediator facilitating the angiogenic activity of sonic hedgehog (Shh).

2. The *IGF2* gene

2.1. Epigenetic regulation of Igf2

Igf2 is widely expressed during murine embryonic development, and is particularly important in placental growth [18]. As with many genes that regulate placental development, *Igf2* is imprinted, or expressed monoallelically, and active only on the paternally inherited allele. *Igf2* is highly expressed in the mouse embryo, but levels decline dramatically after birth; in adult mice, *Igf2* transcripts are detectable only in the choroid plexus and leptomeninges, where expression is biallelic [14]. *IGF2* is also imprinted in humans, but is expressed biallelically in the choroid plexus, leptomeninges, and perhaps the developing retina [19]. However, human *IGF2* is also expressed in the adult, with transcripts arising from an adult-specific promoter [20]. The corresponding region in the mouse *Igf2* gene contains two pseudo-exons and what appears to be a remnant of this adult-specific promoter which may explain why *Igf2* expression ceases after birth in mice but not in humans [21].

Almost all known imprinted genes occur in clusters with one or more reciprocally imprinted genes (reviewed in Ref. [22]). The mouse *Igf2* gene lies on the distal region of chromosome 7 with the oppositely imprinted, non-coding gene *H19*. *Igf2* and *H19* share a set of enhancers that act on either gene, depending on parental origin. In eukaryotic DNA, promoters generally harbor regions dense with CpG dinucleotides, which are targets of methylation. These "CpG islands" are often methylated in inactive promoters. On the paternal chromosome, the *H19* promoter region is methylated and inactive; this methylation and expression

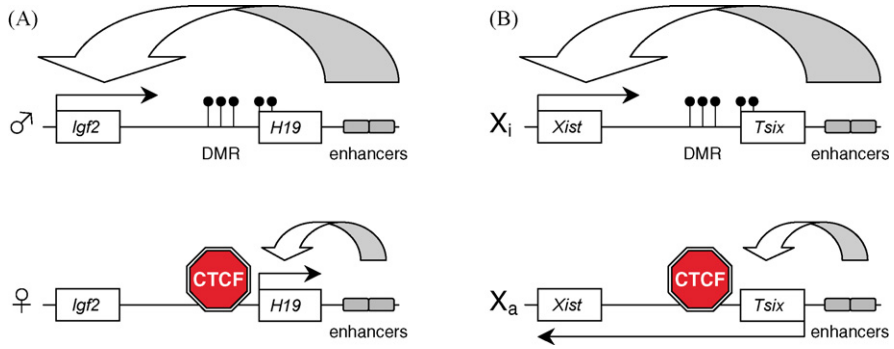

Fig. 1. (A) Model of imprinted regulation at the *Igf2-H19* locus. Adapted from Ref. [25]. (B) Model of allele-specific repression in X chromosome inactivation by CTCF. Adapted from Refs. [26–28]. DMR: differentially methylated region. Lollipops: methylated CpGs. X_i and X_a: inactive and active X chromosomes, respectively.

pattern is passed on when cells divide. Because this inheritance of gene expression patterns is achieved without altering the DNA sequence, it is called *epigenetic*.

The *Igf2* promoter is not methylated on the maternal chromosome, so another mechanism must account for silencing *Igf2*. Several kilobases (kb) upstream of the H19 promoter is a differentially methylated region (DMR) that, when deleted, reactivates *Igf2* on the maternal chromosome [23]. This region, also called the imprinting control region (ICR), was found to harbor binding sites for CCCTC binding factor (CTCF), an insulator protein that demarcates active and inactive chromatin domains (reviewed in Ref. [24]). Methylation of the CG-rich CTCF binding sequence prevents CTCF binding. Thus, on the paternal chromosome, the DMR/ICR is methylated, CTCF is excluded, and the enhancers act on the *Igf2* promoter. Conversely, on the maternal chromosome, CTCF forms a chromatin insulator that blocks the enhancers from activating *Igf2* (Fig. 1A).

2.2. *Igf2* imprinting as a model of allele-specific repression

Murine *Igf2* was the first gene found to be imprinted, and has served as a model of allele-specific gene repression—the most extreme example being X chromosome inactivation, where one X is silenced in each somatic cell of XX female mammals to equalize gene dosage with XY males [29]. *Igf2* imprinting and X chromosome inactivation are the most well-studied mechanisms of epigenetic regulation, and the parallels between these mechanisms give insight into the epigenetic alterations that are abundant in cancer.

X chromosome inactivation generally occurs in a random fashion and silences either X; however, in some mammals and in certain tissues of others, the paternal X is always silenced. In either random or imprinted X chromosome inactivation, the X that is destined to be silenced expresses the non-coding *Xist* RNA, which covers the chromosome and mediates silencing (reviewed in Ref. [30]).

Xist lies in a region called the X inactivation center (XIC) along with another non-coding gene, *Tsix,* which is transcribed antisense to *Xist* and expressed on the active X chromosome [31]. Not long after CTCF was shown to regulate imprinting at the *Igf2/H19* locus, a similar mechanism was found at the *Xist/Tsix* locus. In a region implicated in controlling both random and imprinted X chromosome inactivation, functional methylation-sensitive CTCF binding sites were identified (Fig. 1B). This region was later found to contain developmentally specific enhancers [28] and to be differentially methylated *in vivo* [27]. CTCF has since been demonstrated to control imprinting at several other gene domains, and putative binding sites have been discovered in several other imprinted loci [32]. However, not all imprinted genes contain functional CTCF binding sites. It is proposed that another multifunctional transcription factor, yin yang 1 (YY1), functions as a methylation-sensitive insulator that mediates allele-specific gene activation or silencing at some loci. YY1 has been found to control imprinting at the human *SNURF-SNRPN* locus within the Prader–Willi syndrome and Angelman syndrome locus, and the *PEG3*, *Gnas*, and *Nespas* genes ([33] and references therein). Interestingly, it was reported recently that YY1 is a cofactor for CTCF in X chromosome inactivation [34]. Because both CTCF and YY1 are ubiquitously expressed, it is possible that tissue- and developmentally specific imprinting of *Igf2* is accomplished through a combination of these factors.

The similarities between *Igf2/H19* and *Xist/Tsix* regulation have additional implications for other regulatory mechanisms that may be aberrant in cancer. The X chromosomes initiate silencing after forming a transient interchromosomal complex (reviewed in Ref. [30]). This pairing phenomenon has also been observed with the *Igf2/H19* region, in which CTCF mediates interchromosomal colocalization and induces *trans* effects on a non-homologous chromosome [35]. Interchromosomal pairing may increase the frequency of mitotic recombination, which can account for both heritable and sporadic mutations [36]. Because CTCF mediates interchromosomal pairing of the *IGF2/H19* region, it may very well facilitate such mitotic recombination events. X chromosome inactivation has also drawn attention in the field of cancer research with the recent discovery of X-linked tumor suppressor genes; when mutated, these can lead to hemizygosity in males and skewed X inactivation in females

(reviewed in Ref. [37]). One gene, *FOXP3*, codes for a forkhead family transcription factor that represses the *HER-2/ErbB2* oncogene [38]. Interestingly, the forkhead transcription factors are targets of the PI3-kinase pathway, which is activated by IGF signaling (reviewed in Ref. [39]). The other X-linked tumor suppressor, *WTX*, is frequently inactivated in Wilms' tumor, a disease also associated with disrupted *IGF2* imprinting [40].

X chromosome inactivation can have other implications for *Igf2/H19* regulation as well. There is mounting evidence that non-coding (especially antisense) RNAs regulate allele-specific gene expression (reviewed in Ref. [30]). Multiple sense and antisense transcripts have been detected in the mouse *Igf2* 5′ region, and the major antisense transcript, *Igf2AS,* is paternally expressed and non-coding [41]. An antisense message transcribed from a homologous region near human *IGF2* encodes a putative 273-amino acid protein of unknown function [42]. It remains unclear whether *IGF2AS* regulates *IGF2* or *H19* imprinting; nonetheless, it may have biological importance. In Wilms' tumor, *IGF2AS* is highly expressed and demonstrates sporadic loss of imprinting [42,43]. As stated before, disrupted *IGF2* imprinting is implicated in a number of diseases, and may be attributed to increased gene dosage and subsequent increases in IGF2 signaling, which will be discussed in the following section.

3. The IGF2 protein

3.1. IGF system overview

The IGFs signal primarily through the type I IGF receptor (IGF1R), but there is significant crosstalk between the IGF and insulin systems as certain variants of the insulin receptor (IR) have been shown to bind IGFs (Fig. 2). The alternatively spliced IR-A isoform, which is expressed predominantly during embryogenesis [44], binds insulin and IGF2 (but not IGF1) with high affinity [45]. IGF2 can also stimulate insulin-like metabolic responses by binding the classical IR-B isoform; furthermore, functional heterodimers can form between IGF1R and the IR isoforms (reviewed in Ref. [46]). Thus, tissue-specific effects of insulin and the IGFs may be accomplished through differential expression of the receptors and receptor hybrids. Though IGF1R is activated more efficiently by IGF1 [47], the ability to signal through IR potentially gives IGF2 a broader range of biological functions than IGF1.

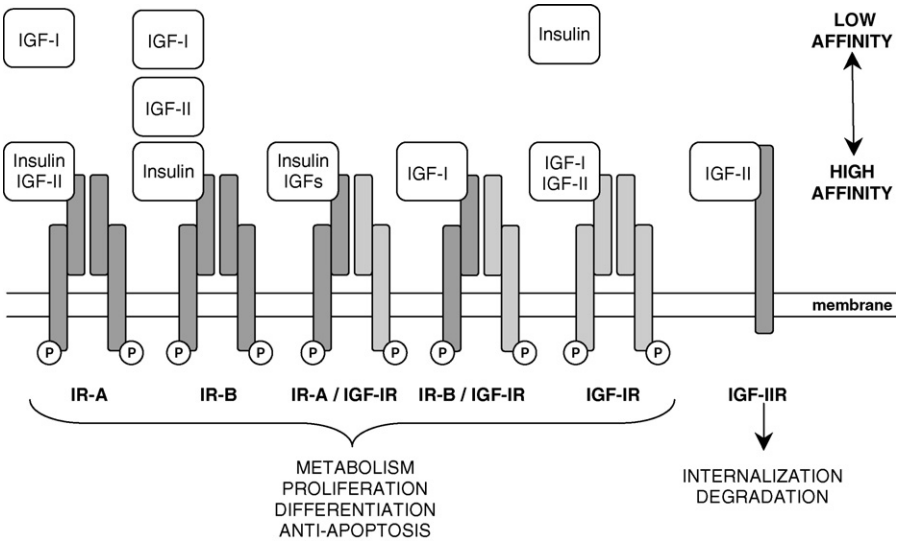

Fig. 2. Overview of the insulin/IGF system. IR exists in two isoforms: IR-A and IR-B. IR-B is responsible for the classic metabolic responses induced by insulin, and also binds IGF1 and IGF2 with low and intermediate affinity, respectively. IR-A has high affinity for insulin and IGF2, and binds IGF1 with low affinity. IGF1R binds the IGFs to stimulate anabolic activity, and also binds insulin at high concentrations. IR-A/IGF1R heterodimers bind insulin and IGFs with similar affinity, whereas IR-B/IGF1R heterodimers bind IGF1 exclusively. IGF2R exclusively binds IGF2 and targets it for degradation. Adapted from Refs. [47,46].

Table 2
IGFBP functions (adapted from Ref. [17])

IGFBP-1	Physiological levels stimulate IGF1 action; molar excess inhibits mitogenic and insulin-like activities of IGF1 and IGF2
IGFBP-2	Inhibitor of IGF-induced DNA synthesis; stimulatory effects on IGF function have also been observed
IGFBP-3	Major carrier of IGFs in serum and modulator of IGF endocrine action; potentiates IGF activity; excessive levels are inhibitory
IGFBP-4	Only IGFBP shown to consistently inhibit IGF action; serum concentration generally low; expression appears to be tissue-specific
IGFBP-5	Inhibitory; association with extracellular matrix (ECM) lowers its affinity for the IGFs, resulting in increased IGF activity
IGFBP-6	Specifically binds IGF2; generally thought to be inhibitory

IGF2 has high affinity for another receptor, IGF2R, and is its principal ligand (Fig. 2). However, IGF2R does not transduce a signal; rather, it serves mainly to limit IGF2 bioavailability by targeting IGF2 for degradation (reviewed in Ref. [48]). Interestingly, the *IGF2R* gene is also imprinted but it is maternally expressed (reviewed in Ref. [16]).

Whereas insulin circulates freely in the bloodstream, the IGFs are found in complexes with the IGF-binding proteins (IGFBPs). Six different IGFBPs have been identified, and each binds the IGFs with significantly higher affinity than IGF1R. The expression patterns of the various IGFBPs differ both spatially and temporally, and they have distinct activities (Table 2). Thus, IGFBPs are important modulators of IGF action, availability, and tissue distribution (reviewed in Ref. [17]). Differential expression of IGFBPs, as well as differential expression of IGF receptors and receptor hybrids, may govern the cell- and tissue-specific actions of IGFs.

3.2. IGF2 in cell growth and differentiation

IGF1 and IGF2 are well known for their mitogenic activities. Almost all cell types express IGF1R, so the IGFs can stimulate growth and differentiation in many tissues (reviewed in Ref. [49]). Upon binding to IGF1R, the IGFs trigger the receptor tyrosine kinase activity, which leads to phosphorylation of itself and its major substrate, the insulin receptor substrate 1 (IRS-1). Phosphorylated IRS-1 can activate the Ras/Raf/MAPK and PI3-kinase/Akt cascades, and depending on the cell type, stimulate proliferation, differentiation, or both (reviewed in Ref. [50]). PI3-kinase activation can lead to anti-apoptotic signals, and components of this pathway are frequently amplified or mutated in cancers (reviewed in Ref. [51]).

The role of IGF2 in muscle development has been studied extensively. IGF2 is upregulated early in MyoD-induced in myocyte differentiation, and signals in an autocrine loop to activate PI3-kinase and Akt [52]. IGF2 inhibition leads to reduced expression of MyoD target genes, which suggests that IGF2 is essential for amplifying and maintaining MyoD efficacy [53]. IGF2 is also essential in bone development, where it promotes proliferation and differentiation of bone cells. Down-regulation of IGF2 most likely accounts for the decrease in bone mass observed with cortisol use [54]. Thus, IGF2 has great therapeutic potential in wound and fracture healing.

Growth in the developing mouse embryo is largely governed by IGF2. When a targeted *Igf2* deletion is transmitted paternally, mouse embryos inherit only the inactive maternal allele and are born runted [14]. Conversely, IGF2 overexpression, achieved by disrupting the inhibitory *Igf2r* [55], by deleting *H19* [56], or by transactivating *Igf2* [57], leads to fetal overgrowth and malformations with characteristics that resemble Beckwith–Wiedemann syndrome (BWS, discussed below).

4. IGF2 and disease

4.1. Loss of IGF2 imprinting

IGF2 is regulated precisely to ensure monoallelic expression in most tissues [19], which emphasizes the importance of gene dosage. Normal development requires accurate expression, and many disorders can be attributed to an abnormally high dose of *IGF2* caused by loss of imprinting (LOI). BWS is one such disease, characterized by fetal and neonatal overgrowth, and is often accompanied by an increased risk of childhood cancers (reviewed in Ref. [58]). BWS patients almost always have mutations in the chromosome 11p15.5 region, a large cluster of imprinted genes that includes *IGF2* and $p57^{KIP2}$ (Fig. 3). Most of these mutations affect imprinting; quite often, biallelic *IGF2* expression and *H19* methylation are observed (reviewed in Ref. [16]). BWS usually occurs sporadically, but in rare familial cases *IGF2* LOI may be caused by deletions of the CTCF binding sites in the maternal *IGF2/H19* ICR [59,60].

Disrupted imprinting is perhaps the most common observation in cancer (reviewed in Ref. [61]), and *IGF2* overexpression is a recurring theme. Wilms' tumor, a childhood cancer of the kidney, is often associated with defects in the WT1 gene, which encodes a transcriptional repressor of *IGF2* [62]. Wilms' tumor is also associated with mutations in the 11p15.5 region that affect *IGF2* imprinting: altered *IGF2* expression accounts for nearly 50% of all cases of Wilms' tumor, and *IGF2* LOI is found in the vast majority (90%) of pathological cases [63]. *IGF2* LOI has also been

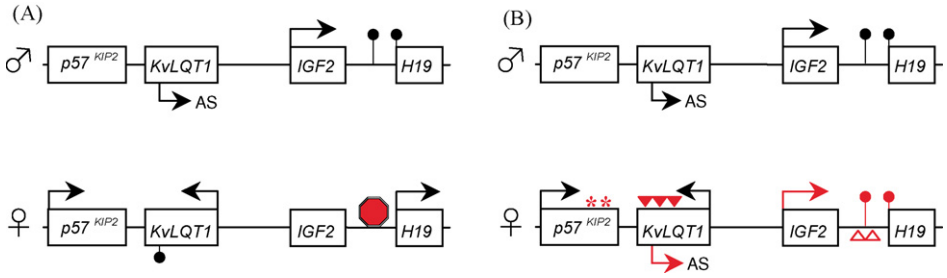

Fig. 3. (A) Normal and (B) BWS gene expression patterns on chromosome 11p15.5. Arrows represent active genes. Lollipops: methylated CpGs. Red octagon: CTCF. Asterisks: point mutations. Filled triangles: translocation breakpoints. Open triangles: deletions. Adapted from Refs. [16,59,60].

observed in many other cancers. Both benign and malignant breast lesions show biallelic *IGF2* expression, and altered imprinting of *IGF2* has been identified in hepatoblastoma, lung cancer, cervical carcinoma, rhabdomyosarcoma, choriocarcinoma, and testicular cancer ([64] and references therein).

The epigenetic mutations associated with cancer, such as aberrant methylation or LOI, may magnify the effects of genetic mutations or even have causal roles. In either case, epigenetic changes have potential value for assessing disease risk and prognosis. In a mouse model of intestinal cancer, where the *adenomatous polyposis coli* (*Apc*) gene is mutated, supplementary *Igf2* LOI increases the incidence of intestinal hyperplasia. The clinical relevance of this is corroborated by the fact that patients with *IGF2* LOI also have an increased risk of developing colorectal cancer [65]. Alterations involving CTCF may also be informative. Elevated CTCF expression levels have been reported in breast cancer, where it is postulated to have anti-apoptotic actions [66]. Gene activation by a CTCF homolog is observed in lung cancer [67,68], and methylation changes in CTCF binding sites have also been reported in osteosarcoma [69]. Because epigenetic changes such as LOI and demethylation are among the earliest events in cancer progression (reviewed in Ref. [70]), assays for epigenetic biomarkers may allow for early detection, prevention, and treatment of cancer.

4.2. IGF2 and other signaling pathways in disease pathogenesis

Igf2 overexpression sometimes occurs without apparent LOI or gene duplication. Other factors, such as sonic hedgehog (Shh), can also transcriptionally activate *Igf2*. Shh is a developmental morphogen involved with patterning and organ specification, and its signaling pathway is mutated in several diseases (reviewed in Ref. [71]). The Shh cascade culminates in the activation of Gli, a transcription factor that induces several target genes (Fig. 4).

Shh has been demonstrated to upregulate *Igf2* both *in vitro* and *in vivo*. When mouse mesenchymal cells are treated with Shh or transfected with *Gli1*, *Igf2* mRNA is upregulated [72]. A *Ptc*-deficient mutation in mice, which results in constitutive Gli activation, increases IGF2 protein levels and also the formation of medulloblastomas and rhabdomyosarcomas [73]. It is not entirely clear how Shh induces *Igf2* expression. Though putative Gli-binding sites have been identified in the mouse *Igf2* promoter [72], it is not known whether these sites are functional, or if they exist in the human VEGF promoter. However, functional Gli sites have been documented in the human *IGFBP-6* promoter [74]. IGFBP-6 specifically binds IGF2 and is generally thought to have anti-proliferative properties. Nonetheless, like most of the IGFBPs (Table 2), IGFBP-6 can have contrasting activities, and has also been shown to be anti-apoptotic and tumorigenic (reviewed in Ref. [75]).

IGF2 itself may provide an oncogenic signal in some systems, such as the mouse mammary gland, where transgenic *Igf2* overexpression induces adenocarcinomas [76]. In mouse models of rhabdomyosarcoma and medulloblastoma, *Igf2* alone is insufficient to generate tumors; however, it can enhance the tumorigenic potential of Shh [73,77]. Interestingly, tumors often overexpress the IR-A variant, which binds IGF2 with high affinity; thus, concomitant IGF2 and IR-A overexpression can potentially generate an autoproliferative loop [30]. Taken together, these observations substantiate the hypothesis that IGF2 can supply the ''second hit'' necessary for oncogene-induced tumors [78].

4.3. IGF2 and angiogenesis

Angiogenesis, or blood vessel growth, is another critical element of tumor progression that may involve IGF2. Oxygen, nutrients, and metabolic wastes can simply diffuse in and out of small tumors, but growth beyond a critical size (1 mm^3) requires a vascular network (reviewed in Ref. [79]). Areas of hypoxia within tumors induce the expression of

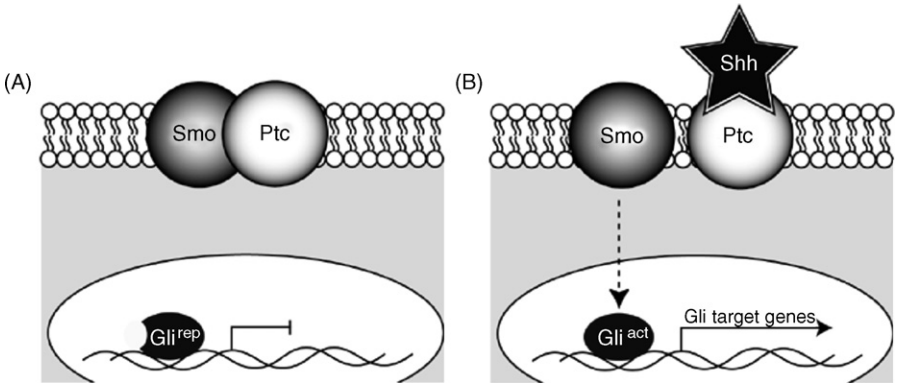

Fig. 4. The Shh signaling pathway. (A) In the absence of signal, the receptor patched (Ptc) is complexed with smoothened (Smo), and Gli exists in a truncated form that acts as a transcriptional repressor [99,100]. (B) When bound by Shh, Ptc releases Smo, which signals to produce a full-length Gli activator protein. Gli target genes include *Gli*, *Ptc*, and genes involved in proliferation and morphogenesis.

angiogenic factors, which prompt an influx of vessels from surrounding tissues. Neovascularization also facilitates the spread of cancer cells to other tissues; thus, there is a correlation between high metastatic potential and tumor vascularity (reviewed in Ref. [80]).

Vascular endothelial growth factor (VEGF) has a central function in both normal and pathological neovascularization, and its expression is upregulated in tumors (reviewed in Ref. [81]). Hypoxia-inducible factors (HIFs) are principle mediators of VEGF upregulation, though VEGF mRNA levels are also increased via message stabilization [82]. Transcriptional regulation also occurs through other *cis* elements in the VEGF promoter, and can be instigated by various growth factors, hormones, and oncogenes (reviewed in Ref. [83]).

Though studies of the IGFs in vascular development are limited, IGF2 may participate in angiogenesis through its ability to upregulate VEGF. In hepatocellular carcinomas cells, hypoxia-induced VEGF expression is increased by IGF2, which is itself upregulated by HIFs [84]**.** Other studies have suggested that IGF2 signaling upregulates VEGF in part by increasing HIF levels [85,86]. Because reciprocal upregulation of IGF2 and HIF has been demonstrated [87], they may act in synergy to induce VEGF expression. Though the mechanisms remain unclear, the ability to induce VEGF accentuates the importance of IGF2 in tumor development.

IGF2 may also be involved in the pathological neovascularization that characterizes proliferative diabetic retinopathy (PDR) and retinopathy of prematurity (ROP). Several studies have implicated IGF1 in retinopathy (reviewed in Ref. [88]), but IGF2 has been largely overlooked—despite reports of 10- to 30-fold more IGF2 in the vitreous of diabetic patients than IGF1 ([89] and references therein). A recent study showed that IGFBP-3 suppressed retinal neovascularization irrespective of IGF1 levels [90], which supported the long-standing notion that IGFBPs can act independently of IGF signaling through IGF1R (reviewed in Ref. [75]). However, the potential contribution of IGF2 needs to be examined—specifically, its interactions with other receptors (such as IR-A variant) and whether these interactions are subject to IGFBP regulation. Clearly, the likely role of IGF2 in retinopathy calls for further exploration.

4.4. IGF2: the missing link between Shh and angiogenesis?

In recent years, Shh has been identified as an angiogenic factor. Studies in zebrafish reveal vascular defects in Shh-mutant embryos [91,92], and place Shh upstream of VEGF signaling during arterial differentiation [93]. The cascades induced by Shh also appear to regulate vessel formation in mammals. In the mouse embryo, Indian hedgehog (Ihh), a Shh homolog, has been suggested to be critical for early vasculogenesis [94,95]. In *Shh*-deficient mice, the developing lung is poorly vascularized [96]; conversely, *Shh* overexpression in the neural tube results in hypervascularization [97]. Shh can also induce angiogenic factors (including VEGF) and promote neovascularization in adult mice [98]. Thus, vessel formation may depend on the ability of Shh to induce VEGF. Though the exact mechanism remains elusive, it may very well involve IGF2, which is a downstream target of the Shh cascade [72] and has a demonstrated ability to synergize with Shh [73,77]. Moreover, IGF2 has also been shown to induce VEGF [84–86]. Thus, IGF2 may mediate the angiogenic effects of Shh, and provide the critical link between Shh and VEGF.

5. Conclusions

Though interest in IGF2 has been somewhat skewed towards the study of gene regulation and imprinting, it is likely to attract attention from other fields as studies implicate IGF2 in an increasing number of diseases. The complexity of IGF2 regulation indicates that overexpression can occur at multiple levels. Since IGF2 is pivotal in many developmental and pathological processes, its multifaceted regulation presents a number of potential therapeutic targets.

Because imprinting defects are now recognized as common in the pathogenesis of cancer, the mechanisms surrounding *IGF2* imprinting are likely to gain interest as well. Perhaps the most thoroughly studied of known imprinted genes, *IGF2* has yielded valuable insight into other epigenetic gene regulatory mechanisms—namely X chromosome inactivation, which also gained significance with the discovery of X-linked tumor suppressors (reviewed in Ref. [37]). These studies highlight the multifactorial nature of cancer, in which IGF2 may have a pivotal role. More importantly, they suggest that imprinting and X inactivation are not just interesting epigenetic phenomena, but have considerable functional relevance.

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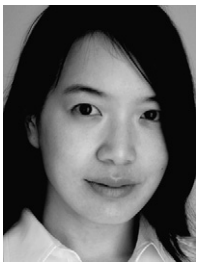

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