Review

Insulin-Like Growth Factor 2 in Physiology, Cancer, and Cancer Treatment

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Abstract

Insulin-like growth factor 2 (IGF2) is a strong mitogenic peptide with an imprinted gene that is primarily involved in fetal development. It is highly expressed in the fetus where it is involved in fetal growth and tissue differentiation. However, postnatally, the expression of IGF2 decreases despite higher expression levels in the blood as compared with that of IGF1. In adults, the physiological function of IGF2 is poorly understood; however, the possibility of a metabolic function exists. Although the expression of IGF2 normally decreases in adults, it is overexpressed in a variety of cancers and associated with increased insulin-like growth factor 1 (IGF1R) receptor and insulin receptor (IR) activity. This subsequently increases the activity of downstream genes such as *AKT*, *FOXO*, and *MAPK*, resulting in enhanced proliferation, survival, and overall worse prognosis in patients overexpressing IGF2. As IGF1R activation has been found in several types of cancers, many different IGF1R-targeted therapies have been clinically evaluated, however, with only limited anti-cancer efficacy. In the present review, the physiological function of IGF2 will be outlined in relation to gene expression, imprinting, and signaling. Additionally, differences in physiological and aberrant signaling of IGF2 in cancer will be summarized.
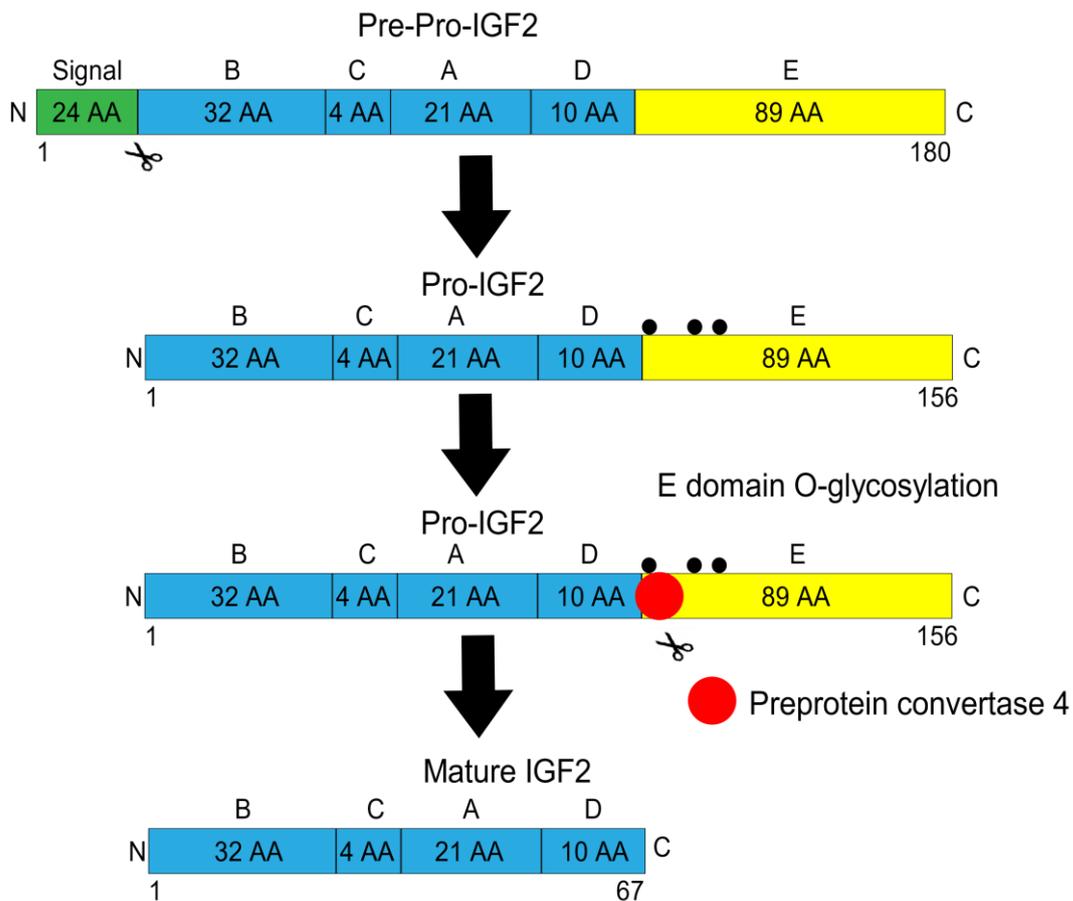
1. Introduction

Insulin-like growth factor 2 (IGF2) is a highly regulated growth factor involved in embryonic development and carcinogenesis and its gene is among the several human imprinted genes. It is a highly conserved peptide partner of the insulin/IGF signaling proteins primarily involved in cell proliferation, growth, and survival by binding to the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R). Other proteins in the IGF-axis include IGF1, IGF-binding proteins (IGFBP), IGF2 mRNA binding proteins (IGF2BPs), and the IGF2R (also known as a cation-independent mannose-6-phosphate receptor).

Compared with levels of IGF1 in mammals, higher levels of IGF2 are expressed in the serum [1-3]. Moreover, it is primarily expressed in prenatal life. The function of IGF2 in the prenatal life is better understood; it regulates placentation through the AKT pathway [4] and skeletal muscle differentiation [5]. Additionally, the administration of IGF2 in rats has been reported to significantly increase the weight of the stomach, intestine, liver, and pancreas, whereas the weight of the heart, lungs, and kidneys remained unaffected, indicating the role of IGF2 in the development of these organs [6]. Although IGF2 is more abundant than IGF1 in the adult serum, the physiological function of IGF2 in adults is poorly understood in comparison with that of IGF1. Evidence suggests that IGF2 plays a role in the metabolism of certain tissues such as skeletal muscles, fat, and bone. However, the exact metabolic function of IGF2 in these tissues remains unclear [7]. Additionally, mouse studies revealed that IGF2 expression ceased after the birth, indicating that this postnatal function of IGF2 was not highly conserved between different species. Furthermore, one of the gene promoters, not present in mice, is known to be likely regulating the postnatal *IGF2* expression in humans [8-11].

Although IGF2 and IGF1 are considerably similar proteins, they function differently. While deletions and homozygous mutations in *IGF1* resulting in IGF1 deficiency have been described in patients of young and adult age [12-14], no such cases have been reported for deletions in *IGF2* gene, suggesting the necessity of IGF2 for the survival in the prenatal development [15]. Additionally, in mice, heterozygous deletion of IGF2 leads to a higher reduction in the birth weight (60%) [16] than that caused by the heterozygous deletion of *IGF1* (10–20%) [17], further indicating the importance of IGF2 in embryonic development. Furthermore, postnatal IGF1 expression is induced by the growth hormone (GH) signaling in the liver, whereas IGF2 expression in human adults is not regulated by GH. IGF2 is produced in the choroid plexus, leptomeninges of the brain, liver, adipose tissue, female reproductive organs, and placenta [18-20], highlighting the differences in the signaling and expression between IGF1 and IGF2.

However, in a similar fashion as Similar to IGF1 and insulin, IGF2 is translated as a pre-pro-protein (180 amino acids) in humans. Depending on the tissue, age, and stage of prenatal development, it is transcribed through the activation of one or more of five promoters. Which promoter is responsible for the expression in which tissue is not well known in humans. However, Ghanipoor-Samami et al. demonstrated the promoter activity of IGF2 in bovine tissues,

postulating that bovine IGF2 promoters and expression are more similar to that of human IGF2 than to that of, for instance, mice where promoter 0 (P0) is a placenta-specific promoter, contrary to that observed in humans and bovines [21]. Furthermore, P0 seems to be inactive in the bovine placenta, whereas it accounts for approximately 10% of the placental IGF2 expression in mice [21, 22]. This could be explained by the finding that the recruitment of different ZFPs in murine and human cells to P0 of IGF2 regulates its transcription [22]. IGF2 is translated as a pre-pro-protein containing six subdomains, namely A to E, and a 24-amino acid signal peptide that is post-translationally processed to release the mature form of IGF2 (Figure 1).

Figure 1 Mature IGF2 production from the pre-pro-protein and the pro-protein. The red circle denotes the preprotein convertase 4 (PC4) while the black dots denote glycosylated amino acids on domain E.

The 24 amino acids of the signal domain are cleaved, creating pro-IGF2, which is subsequently O-glycosylated on the E domain; the E-domain promotes further processing of the pro-peptide [23]. The 89 amino acids of the E domain of pro-IGF2 are next cleaved through proteolysis by proprotein convertase 4 (PC4 coded by *PCSK4* gene), releasing the final product IGF2, which, compared to insulin, is a monomer consisting of a single amino acid chain. The protein is then secreted into the bloodstream where it exerts its function on growth and development [24].

Besides regulating fetal development, IGF2 plays important role in stem cells. For example, induced IGF2 knockouts significantly reduced the number of neural stem cells present in the subgranular zone and the subventricular zone of the mouse brain. Furthermore, this decrease

resulted in neural deficits, and mice with knocked-out IGF2 took longer to complete the Morris water maze when compared with control mice [25]. Additionally, reduced body weight along with the degradation of the intestinal villi was observed in the IGF2 knockout mice, suggesting the role of IGF2 in maintaining stem cell populations in the intestine. Indeed, transit-amplifying cells were abundant in wild-type (WT) intestinal crypts; however, these were significantly decreased in the IGF2 knockout mice owing to reduced self-renewal capacity of intestinal stem cells [25]. Interestingly, this occurs independently of IGF1R signaling with the insulin receptor A (IRA) being the main receptor mediating the maintenance of neural stem cell populations [26].

Apart from its physiological function, IGF2 is involved in multiple diseases. For instance, overexpression of IGF2 can cause Beckwith–Wiedemann syndrome (BWS), an overgrowth disorder present from the birth resulting in microcephaly, macrosomia, and macroglossia [27]. The overgrowth results from an excess of IGF2 due to uniparental disomy (UPD). Because IGF2 is an imprinted gene, it is physiologically expressed only from the paternal chromosome. UPD can be found in 10 to 20% of cases of BWS, resulting in two paternally derived copies of IGF2 and thus its overexpression [28, 29]. Additionally, the aberrant expression of IGF2 has been indicated in several metabolic disorders, such as metabolic syndrome and coronary heart disease [30]. Moreover, IGF2 might play a role in the progression of diabetic nephropathy in diabetes mellitus types 1 and 2 [31]. However, no conclusive causative link has been established between IGF2 and these disorders. In mice, however, transgenic overexpression of IGF2 prenatally as well as in adulthood resulted in severe cardiac abnormalities such as cardiomegaly and enlargement of the left ventricle [32]. Furthermore, overexpression of IGF2 is strongly involved in cancer and its development owing to the mitogenic potential of IGF2. Several cancers overexpress IGF2 (Table 1) that help cancer cells to proliferate and survive, thus contributing to cancer progression [33].

Table 1 Various cancers with upregulated IGF2 expression reported in the literature.

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Hepatocellular carcinoma [34]	Hepatoblastoma [35]
Glioblastoma [36]	Bladder cancer [37]
Breast cancer [38]	Pancreatic carcinoma [39]
Tongue carcinoma [40]	Osteosarcoma [41]
Acute myeloid leukemia [42]	Rhabdomyosarcoma [43]
Esophageal cancer [44]	Ewing sarcoma [45]
Colorectal cancer [46]	Pleural solitary fibrous tumors [47]
Endometrial cancer [48]	Ovarian cancer [49]
Adrenocortical tumors [50]	Prostate cancer [51]

(x)* reference for listed cancer types.

Patients with BWS overexpressing IGF2 have a higher baseline chance of developing cancer, in particular, Wilms' tumor [27], suggesting that IGF2 is involved in the development of cancer. Indeed, upon examination of family histories, IGF2 overexpression was found to correlate with an increased risk of developing several cancers such as colon cancer and breast cancer [52]. Additionally, cancer patients with tumors overexpressing IGF2 experience rapid disease progression [53], shorter event-free survival after treatment [54], and worse overall survival [55], highlighting the importance of IGF2 signaling in cancer. In the present review, IGF2 signaling will

be characterized in the physiological context as well as cancer to outline the differences between its signaling in physiology and cancer with respect to splicing, imprinting, downstream signaling, and cancer treatment.

2. Production of IGF2

2.1 Promoters and Gene Isoforms

The *IGF2* gene is located on chromosome 11p15.5 and its transcription can be initiated from five different promoters (Figure 2). Depending on which promoter transcription is initiated, the *IGF2* RNA has a different 5'-UTRs; however, the final translated protein remains the same. The *IGF2* gene consists of nine exons (~7000 bp), of which only exons 7, 8, and part of exon 9 encode for the pre-pro protein of IGF2. The initiation of transcription from different promoters includes different non-coding exons into the mRNA. Promoter 1 (P1) includes exon 1, which lies furthest from the translated region, exon 3, and a part of exon 2. In contrast, P0 includes complete exons 2 and 3, whereas promoter 2 (P2), encoding for the long isoform of *IGF2*, includes exon 4a in the UTR and can either include or exclude exon 4b. Furthermore, promoter 3 (P3) includes exon 5, whereas promoter 4 (P4) includes the small exon 6 to generate the mRNA with the shortest 5'-UTR. Activation of P2 can result in a different protein isoform of IGF2 through inclusion and translation of exon 4b, resulting in the long isoform of IGF2 with an expansion of the N-terminal part of the protein isoform [56]. Similar to P0, P1, P3, and P4, the normal-sized translated product of IGF2 can also be obtained from P2 expression, albeit with different inclusion of non-coding exons into the mRNA [21].
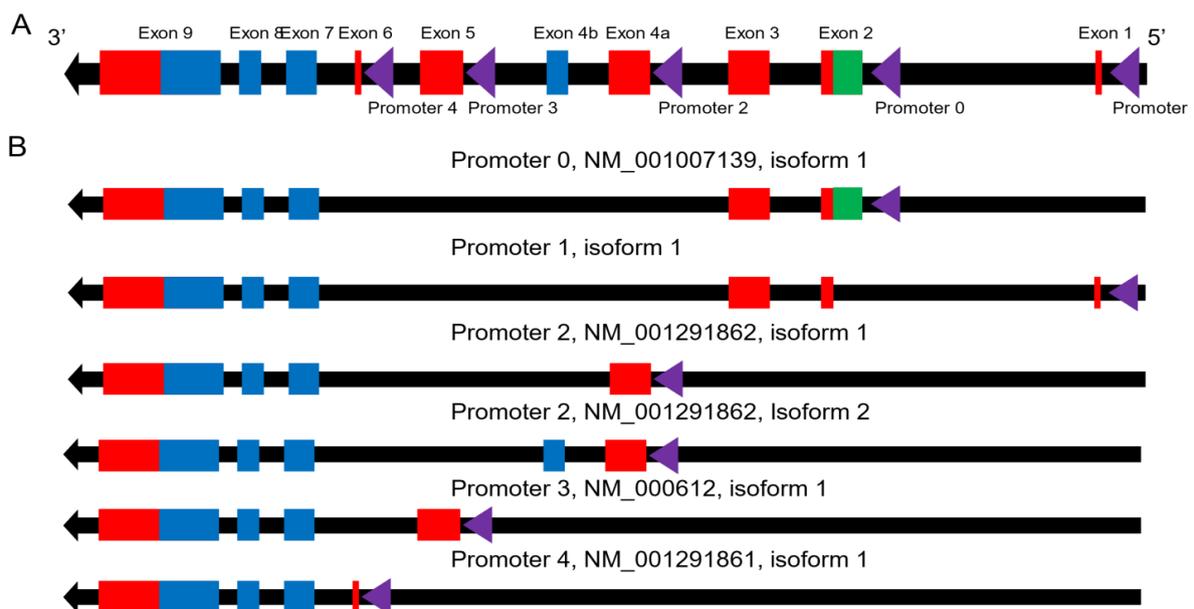
Figure 2 Schematic view of IGF2 isoform transcription from different promoters. A) Squished view of *IGF2* gene with all promoters and exons. B) IGF2 gene isoforms showing each promoter and its induced transcription. Black line: intronic and intergenic regions, boxes: exons, triangles: promoter. Red boxes: untranslated regions (UTR), blue boxes: coding exons; green box: exon 2 UTR part exclusively initiated by promoter 0. The sizes of exons and introns are proportional to bp (base pair) size.

The promoters of *IGF2* are differentially activated during embryonic development. While P2 to P4 are active in the embryonic tissues [57], P1 is exclusively activated in adult tissue [58]. Generally, IGF2 expression decreases after the natal period although the activity of P1 increases throughout life after birth. In contrast, the P4 activity displays an age-dependent decrease after reaching the peak activity in fetal life. P2 and P3 reach the highest activity shortly after birth and stay roughly stable after 18 months. Additionally, P1 has been called the liver-specific promoter, as its activity was only found in the adult liver [59-61]. However, this point has become contentious over time as the activity of P1 has been shown in other tissues as well [62]. Furthermore, in contrast to mice, where IGF2 expression ceases after birth, P1 is a promoter not shared with murine species and drives IGF2 expression postnatally [8-10]. The activity of P0 is primarily found in the fetal skeletal muscles and placenta [63] early during the embryonic and placental development. Nonetheless, later in fetal development and in adult life this promoter is active in a variety of different tissues, albeit with lower activity than P2 to P4 in the prenatal life or P1 in adult life [64] (Table 2).

Table 2 Overview of IGF2 expression from different promoters in pre- and postnatal life.

|	**Prenatal**	**Natal**	**Adult**
Promoter 0	Primarily expressed: Fetal skeletal muscle/ Placenta	Low expressed	Low expressed
Promoter 1	-	-	Liver/ Myometrium
Promoter 2	+	Highest expression shortly after birth	Stable expression after 18 months
Promoter 3	+	Highest expression shortly after birth	Stable expression after 18 months
Promoter 4	Peak expression	Age dependent decrease	Age dependent decrease

(-) = not expressed, (+) = widely expressed.

The *IGF2* gene is also involved in the expression of transcription products other than *IGF2* mRNA. It is located adjacent to the insulin gene and *Insulin-IGF2* readthrough transcripts have been reported to code for a fusion protein of 62 amino acids of the insulin gene and 138 amino acids of the *IGF2* gene. This protein is mainly expressed in the pancreatic β-cells and might act as an autoantigen involved in the development of diabetes type 1 [65]. Additionally, a long non-coding RNA readthrough of the INS-IGF2 gene has been shown to promote cell proliferation and migration in non-small cell lung cancer (NSCLC) [66] and is upregulated in insulinomas [67]. Furthermore, the gene encodes a miRNA-483 in the intron between exons 8 and 9, which can selectively enhance the transcription from fetal *IGF2* promoters, thus regulating it [68]. Lastly, the *IGF2* gene encodes an antisense gene, *IGF2-AS*, which is present on the opposite strand of the

IGF2 gene. The function of *IGF2-AS* is not yet completely understood. The *IGF2-AS* is considered to be non-coding and also sources such as GeneCards [69] and NCBI RefSeq [70] describe this product as non-coding. However, Okutsu et al. demonstrated that the *IGF2-AS* transcript contains an open reading frame that encodes for a putative 273 amino acids long peptide in Wilms' tumor [71]. Nevertheless, it appears that even after the description of the putative *IGF2-AS* protein by Okutsu et al., this protein has not been discovered in any tissue or cancer. Therefore, most likely, it only functions as a long non-coding RNA (lncRNA). However, the exact function of this lncRNA remains elusive as it has been shown to function as an epigenetic tumor suppressor in prostate cancer [72] but as an oncogene inducing ERK/MAPK activity in hepatocellular carcinoma [72].

2.2 Imprinting of IGF2

IGF2 is one of the several human imprinted genes. It is maternally imprinted and thus monoallelically expressed, except in a selected few tissues such as the choroid plexus, leptomeninges, and the developing retina [73]. Imprinting generally occurs in clusters on chromosomes under the control of DNA elements called imprinting control regions (ICRs) [74]. *IGF2* is located in such an imprinting cluster on the short arm of chromosome 11, along with the *H19* gene. The *H19* gene codes for a 2.3 kb non-coding RNA [75] that functions as a tumor suppressor [76]. It is expressed prenatally and down-regulated postnatally, and imprinted in tandem with the *IGF2* gene (Figure 3).
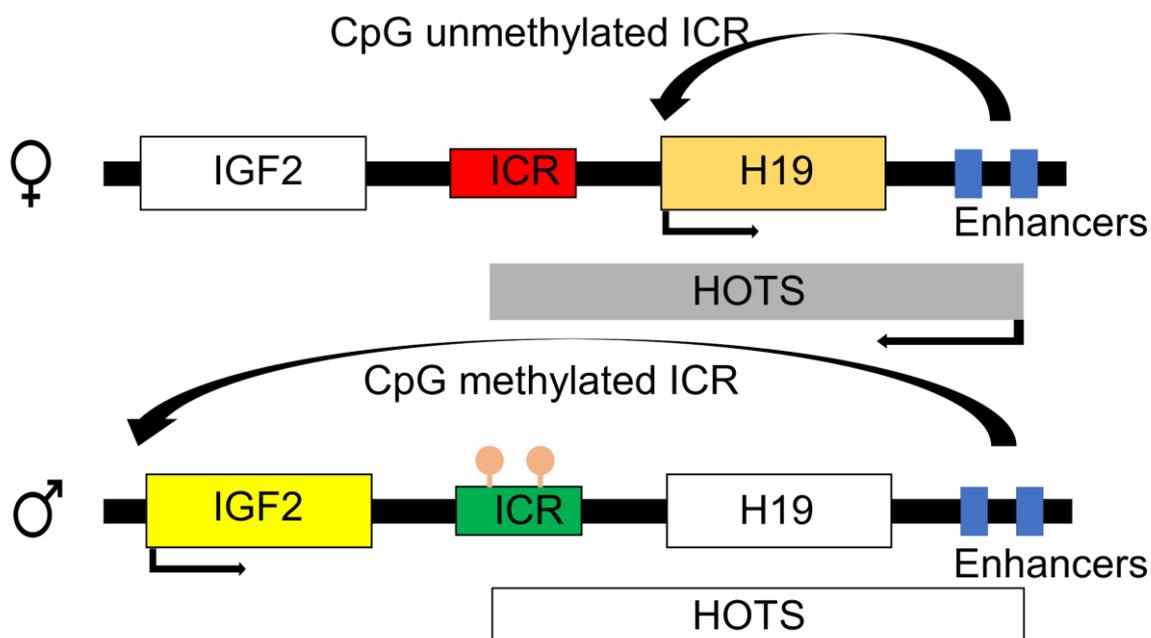
Figure 3 Imprinting of IGF2 and H19 [77]. The unmethylated ICR (red) on the maternal allele is blocking the signal from the enhancers leading to H19 activation, while the methylated ICR (green) on the paternal allele is allowing the enhancers (blue) to stimulate expression of the IGF2 gene downstream of ICR. Genes in yellow and white boxes depict expressed and suppressed genes, respectively. Arrow denotes the gene expression that is stimulated by the enhancers. The grey gene is H19 Opposite Tumor Suppressor (HOTS) which is transcribed only from the maternal chromosome and lies on the antisense strand of H19.

The proposed mechanism for *IGF2* and *H19* imprinting is based on the hypothesis that both *IGF2* and *H19* are controlled by the same enhancer regions on chromosome 11. However, based on the methylation status of CpGs in the ICR, either *IGF2* or *H19* expression could be induced by activation of the enhancer regions. When the ICR is un-methylated, it acts as an insulating signal for the activity of the enhancers, causing transcription only downstream of the ICR and thus of the *H19* gene. However, when ICR is methylated, the enhancer signal stimulates the expression of the gene further upstream of it, thereby inducing *IGF2* transcription as compared with that of *H19*. During imprinting, the ICR is only methylated on the paternal chromosome, resulting in monoallelic expression for both *H19* and *IGF2* from the maternal and the paternal chromosomes, respectively. The mechanism through which the unmethylated ICR acts as an insulator is incompletely understood. However, Hiroyuki et al. proposed a model in which chromosome looping is affected by the methylation of ICR, bringing either *H19* or *IGF2* in close proximity to the enhancer regions based on the ICR methylation status [78].

Furthermore, *IGF2* is not imprinted in every tissue during all stages of life. As mentioned earlier, *IGF2* is bi-allelically expressed in the developing retina [73]. Additionally, not all the previously described *IGF2* promoters are imprinted. While P2 to P4 are imprinted in the manner described above, owing to their close proximity to the CpG-rich ICR, P1 is located more than 20 kb upstream and therefore escapes imprinting in several tissues, including leptomeninges, the choroid plexus, and the liver. It is therefore responsible for the non-imprinted expression of *IGF2* [79]. It appears that the biallelic expression of *IGF2* observed in these tissues and the developing retina results from the P1 activity that escapes imprinting rather than a lack of imprinting. Whether the expression of *IGF2* is mono- or bi-allelic in certain tissues, therefore, depends on which promoter drives its expression. Additionally, this would subsequently indicate that P1 signals in tissues besides the liver, which bi-allelically expresses *IGF2*, are attributed to the involvement of P1 in bi-allelic expression.

Because *IGF2* expression is controlled by imprinting of the gene, UPD can cause aberrations in its expression. UPD causes two copies of a chromosome (segment) of either paternal or maternal to be present instead of one copy from each parent. The dysregulation of *IGF2* through UPD has been reported to be associated with several disorders. For example, in BWS, 10% to 20% of cases present with two parentally derived copies of the *IGF2* locus 11p15. In these individuals, not only the overexpression of *IGF2*, but also the decreased levels of the maternally expressed gene *CDKN1C* contribute to the BWS phenotype, resulting in somatic overgrowth and an increased risk of several cancers [28].

2.3 Post-Transcriptional Control of IGF2 mRNA

Other regulatory units of IGF2 signaling include the IGF2 mRNA binding proteins (IGF2BPs). This family of proteins consists of three members, namely, IGF2BP1, IGF2BP2, and IGF2BP3 [80]. These proteins can bind to several RNA transcripts, including IGF2, thereby stabilizing and protecting these transcripts from degradation. Additionally, IGF2BP can stimulate the transcription of the bound mRNA [81]. Like IGF2, IGFBPs are generally highly expressed during fetal development, and thus involved in fetal development through stimulating the translation of several proteins including IGF2. In adults, IGFBP expression is repressed although it can be re-expressed in cancer [81]. For example, *IGF2BP2* is overexpressed in a variety of cancers, including hepatocellular

carcinoma (HCC), glioblastoma, colon cancer, and breast carcinoma [82]. Furthermore, *IGF2BP* is overexpressed in cancer stem cells, a group of cancer cells that can initiate tumor growth and are considered responsible for cancer recurrence and metastasis [83, 84].

2.4. Post-Translational Modification of IGF2

The different forms of IGF2 are modified after translation to facilitate the maturation of the IGF2 peptide from pre-pro-IGF2. The O-glycosylation of pro-IGF2 on the E-domain of the peptide stimulates proteolytic cleavage by pre-protein peptide convertase-4 (PC4) of the protein, first at Arg-104 and then at Arg-68, to yield the 67 amino acid-long mature protein (Figure 1) [24]. The functional importance of this process is highlighted during human development, where altered processing of pro-IGF2 may lead to intrauterine growth restriction (IUGR). To support this, increased levels of pro-IGF2 have been reported in placental tissue and serum of patients carrying IUGR fetuses [24]. Pro-IGF2 contains multiple potential glycosylation sites, of which the site at Thr-75 is known to be used for glycosylation [85]. After the production of pro-IGF2 in the endoplasmic reticulum, the protein is transported to the Golgi-apparatus, where N-acetyl galactosamine residues are attached to its E-domain in the cis-Golgi compartment, starting the glycosylation process. Subsequently, sialic acid side chains are attached to the N-acetyl galactosamine in the trans-Golgi compartment, and finally, the oligosaccharide side chains mature in the trans-Golgi network, resulting in endo-proteolysis and releasing mature non-glycosylated IGF2 [85]. These mature IGF2 can be secreted into the bloodstream. Disruption in this system is associated with several diseases such as the aforementioned IUGR. In addition, abnormalities in PC4 expression have been indicated in cancer [86] and infertility [87, 88]. Pro-IGF2 has also been shown to be a potent activator of IGF2 signaling in tumors [89]. This not only contributes to cancer progression in patients but also to IGF2-related symptoms. Overexpression and abnormal processing of IGF2 can also cause paraneoplastic syndromes. For instance, in solitary fibrous tumors with overexpression of IGF2, the paraneoplastic Doege-Potter syndrome has been described, wherein the IGF2 produced by the tumor causes severe hypoglycemia [90]. A case report of a patient with a solitary pleural tumor showed decreased amounts of *PC4* mRNA, resulting in reduced conversion of pro-IGF2 into mature IGF2 by PC4. This resulted in the increased secretion of pro-IGF2 and a corresponding increase in IGF2 activity, which subsequently caused non-islet cell tumor hypoglycemia and unconsciousness [91]. These findings highlight how the disruption in PC4 expression or in the glycosylation process of IGF2 can contribute to cancer progression as well as systemic symptoms related to cancer.

2.5 Bio-Availability of IGF2

Other proteins that regulate the bioavailability of IGF2 are the IGF-binding proteins (IGFBPs). When IGF2 circulates freely in the bloodstream, it is unstable and can be quickly degraded. The IGFBPs are a group of six proteins with highly similar amino acid sequences that can bind to and stabilize IGFs in the bloodstream. Among these, IGFBP-2, -3, and -5 commonly bind to and stabilize IGF2, thus increasing its bioavailability [39]. Generally, IGFBP-3 binds IGF2 more commonly than other IGFBPs, binding ~90% of IGF2 in the bloodstream [92]. Furthermore, IGFBP can modulate the activity of IGF. Upon the addition of either IGFBP-3,-4, or -5 *in vitro*, the IGFBPs bind to IGF2 and downregulate its activity by blocking its binding to target receptors [92]. This indicates that IGFBP

not only increases the bioavailability of IGF2, but also modulate its activity. The IGFBP is also differentially expressed during development. The expression of IGFBP1 is restricted to the liver, while IGFBP2 is mainly expressed in the ectodermal and endodermal-derived tissues and IGFBP3 is primarily expressed in a subset of mesenchymal cells during development. This suggests different roles for each IGFBP in the trafficking of IGF2 during the life of the fetus [92].

3. IGF2 Signaling

The mechanism of signaling of IGF2 has been extensively studied. IGF2 primarily transmits signals through the receptor dimers of IGF1R and the Insulin Receptor (IR). Two IR variants, resulting from the splicing out of exon 11, are expressed throughout the body. The variants, IRA and IRB, that lack or retain exon 11 of *INSR*, respectively, elicit different downstream signals. While the IRB is involved in the glucose metabolic function of IR signaling, IRA is associated with embryonic development and cell proliferation [93]. Of the possible combinations of receptor dimers, IGF2 binds with the highest affinity to the IRA homodimer, the IGF1R homodimer, and the IGF1R/IRA heterodimer [26, 94-96], which is unsurprising considering the mitogenic and growth potential of the IGF2 peptide, the mitogenic downstream signaling of these receptor dimers, and the limited effect of IGF2 on metabolism.

Upon binding with the ligand, the receptor dimers trans-phosphorylate their intracellular kinase domains and activate intracellular signaling. In the case of IGF2, the dimers IRA and IGF1R recruit and phosphorylate Insulin Receptor Substrates (IRS), which can activate AKT, FOXO, and MAPK pathways. This, in turn, induces cell proliferation and survival, and thus plays important physiological and pathophysiological roles in prenatal development and cancer, respectively [33, 97]. However, different IRS proteins activate different downstream signaling pathways. While the phosphorylation of both AKT1 and AKT2 is induced by IRS2, IRS1 induces the phosphorylation of only AKT2. In addition, IRS2 is more potent in inducing the phosphorylation of ERK and MAPK than IRS1 [98]. Furthermore, the differentiation of adipocytes from fibroblasts was more profoundly inhibited by IRS2 knockdown than IRS1 knockdown, suggesting differences in the downstream pathways activated by either IRS [99]. Moreover, several different functional kinases are affected differently by knockdown of IRS1 or IRS2 [100], highlighting the importance of differential downstream activation through IRS1 and IRS2 in physiology. Regarding IGF2 signaling, different IGF-related ligands binding to the receptor dimers can recruit and activate different IRS proteins. For instance, in myoblasts, the IR preferentially phosphorylates IRS2, but not IRS1, upon binding with IGF1 [101]. Similarly, upon activation of IRA by IGF2, although both IRS1 and IRS2 are phosphorylated, IRS2 is phosphorylated more than IRS1 [102]. However, little is known about the preferential IRS recruitment and downstream phosphorylation of the IGF1R upon IGF2 binding.

Apart from the IR and IGF1R, IGF2 can bind to the IGF2R, which is also called the monomeric cation-independent mannose-6-phosphate receptor. However, this receptor lacks a functional kinase domain and is generally considered to inhibit the IGF2 function. Through the binding of IGF2, the IGF2R reduces the bioavailability of IGF2, and therefore can act as a growth inhibitor, due to which it is considered to be a tumor-suppressor [103-105]. In addition, the extracellular domain of the IGF2R can dissociate from the cell membrane after proteolytic cleavage and circulate through the bloodstream, bind to IGF2, and aid in its degradation. IGF2 signaling needs to

be tightly regulated, as aberrant IGF2 activity is indicated in several diseases including cancer [106], thus highlighting the importance of the IGF2R in physiology.

4. IGF2 in Cancer

4.1 IGF2 Signaling in Cancer

IGF2 plays a major role in cancer as a mitogenic growth factor that transmits signals through IGF1R and IRA receptors. In several cancer types, the IGF1R is often overactive and overexpressed causing increased proliferation and survival of cancer cells due to the upregulation of downstream pathways, while the receptor is rarely mutated [107, 108]. Furthermore, the activation of IGF1R has been found to be necessary and sufficient for malignant transformation of certain cell types such as fibroblasts in the *in vitro* transformation to Ewing sarcoma [109]. As both IGF1R and IGF2 can be overexpressed in cancer cells, IGF2 can transmit signals in an autocrine loop, wherein the IGF2 secreted by the cancer cell can bind to the IGF1R on the same cell. This subsequently upregulates AKT, FOXO, and MAPK pathways, promoting proliferation and survival of the cancer cells. Similarly, paracrine IGF2 signaling has also been indicated in cancer, where the stroma of cancer cells secretes IGF2 and stimulates carcinogenesis [110]. In addition, overexpression of IGF1R and IGF2 was reported to be associated with reduced overall survival (OS) and poor prognosis [27, 53, 54, 111]. However, IGF1R expression is not always correlated with poor prognosis. In luminal-B breast cancers, overexpression of IGF1R has been correlated with the better OS [112]. Moreover, luminal-A and -B breast cancer patients with reduced expression of IGF1R showed lower OS [113]. In contrast, in triple-negative breast cancer (TNBC), expression of IGF1R was associated with worse disease-free survival [114, 115]. It appears that the expression of hormone receptors in breast cancer cells affects the function of IGF1R. *In vitro*, it has been shown that IGF1R signaling inhibits the invasiveness of breast cancer cells, but only when the estrogen receptor is also expressed [116]. This might explain why the reduced expression of IGF1R is correlated with reduced OS in hormone receptor-positive breast cancer patients due to increased aggressiveness and invasiveness of tumor cells, which is not the case in TNBCs. These results highlight the importance of not only tissue-specific analysis but also other biological parameters (such as estrogen-receptor status), in investigations.

Furthermore, the pro-IGF2 can be secreted by tumors as well as IGF2. The pro-IGF2 has similar binding affinities to IGF1R and IRA as mature IGF2 but has decreased affinity to IGF2R. Therefore, pro-IGF2 remains active regardless of the level of IGF2R, while IGF2 is sequestered and inhibited. The expression and production of mainly pro-IGF2 tumors can avoid the downregulation of IGF2R-mediated IGF2 signaling and promote cancer progression [89]. Indeed, the expression and secretion of pro-IGF2 instead of mature IGF2 seem to be mediated by the decreased expression of the PC4 protein, which is responsible for the cleavage of pro-IGF2 [86, 91].

4.2 Overexpression of IGF2 in Cancer

Several types of cancer overexpress IGF2. This increased expression is often mediated by a loss of imprinting (LOI) of the ICR, such that it is methylated on both chromosomes, causing biallelic expression of *IGF2* [51, 117, 118]. The LOI is usually mediated by decreased binding of the enhancer-blocking element CCCTC-binding factor (CTCF) to the ICR region on the unmethylated

chromosome. This subsequently allows methylation of the CpGs in the previously unmethylated ICR, thus disrupting the imprinting of maternal *IGF2* and expression of biallelic *IGF2* [119]. In addition, LOI of *IGF2* results in loss of expression of *H19*, a previously described tumor suppressor [120], thus driving carcinogenesis through IGF2 expression as well as the loss of *H19*. However, the replacement of *H19* RNA in cancer cells shows no phenotypic effect, meaning that the exact function of the *H19* RNA remains unclear. Nevertheless, the *H19* gene locus also contains an antisense protein called the H19 opposite tumor suppressor (HOTS). Overexpression of *HOTS* inhibits the growth of the rhabdoid tumor, rhabdomyosarcoma, and choriocarcinoma, while its silencing increases the growth of tumor *in vivo* [121]. Furthermore, *HOTS* is imprinted in the same manner as *H19* and *IGF2*, wherein LOI of the 11p15 locus results in loss of expression of *H19* as well as *HOTS* [121]. Therefore, carcinogenesis of the LOI of the 11p15 locus is not only mediated by the overexpression of IGF2, but also the loss of expression of *HOTS* and *H19*. Another mechanism that ultimately results in LOI involves the loss of heterozygosity (LOH), either copy number-neutral (CN-LOH) or due to allelic loss. LOH disturbs the expression of *IGF2/H19/HOTS* and promotes the growth and survival of tumors through similar mechanisms. As LOH may involve a larger region on chromosome 11, the effects of LOH can also involve other imprinted genes, such as CKDN1C [122] and WT1 [123], in this gene region. LOH of the 11p15.5 locus that leads to disturbed IGF2 expression has been frequently observed in rhabdomyosarcoma (RMS) [124]. Although LOH has been mainly observed in both translocation-negative and -positive RMS, LOH of the 11p15.5 locus was identified as an early driving alteration in fusion-negative RMS [125]. LOH of the locus has also been reported in Wilms tumor [123] and hepatoblastomas [35].

However, LOI and LOH, by themselves, do not always explain the increased expression of IGF2 that is observed in tumors. Additional regulatory pathways of IGF2 are required to explain the significant increase of IGF2 expression in some tumors. Similarly, IGF2 expression is higher in fetal tissues than adult tissues, although it is mono-allelically expressed in both, indicating that other regulatory elements are involved. Different transcription factors are able to induce *IGF2* transcription through binding to the ICR close to *IGF2*. One such factor, ZFP57, normally only expressed in embryonic stem cells, induces *IGF2* expression, and is overexpressed in pancreatic, esophageal, and breast cancers [126]. Likewise, the transcription factor E2F3 can directly increase the expression of *IGF2* by binding to fetal *IGF2* promoter sequences, primarily P3. Therefore, the downregulation of E2F3 is necessary for decreasing the postnatal *IGF2* expression [64]. E2F3 is the only E2F transcription factor that has been shown to be upregulated in certain cancers, resulting in increased *IGF2* expression in these cancers, for example, bladder and prostate cancers, wherein samples with E2F3 overexpression also showed overexpressed *IGF2* [64]. Furthermore, the overexpression of inhibitor of DNA binding 1 (ID1) has been associated with increased IGF2 secretion in cell lines [44] and the knockdown of achaeta-scute complex-homolog 1 (ASCL1) increased *IGF2* expression in neuroblastoma cells [127], illustrating the complexity of the overall regulation of *IGF2* gene expression. Other than proteins affecting transcription, Weischenfeldt *et al.* showed that specific rearrangements of the IGF2 locus can drive the overexpression of IGF2 through super-enhancer hijacking. Tandem duplications in the *IGF2* gene placed IGF2 under the transcriptional control of a super-enhancer through chromatin looping, causing *IGF2* overexpression [128].

Apart from imprinting, *IGF2* can also be amplified. This amplification was reported in 7% of colorectal cancers in a study. Amplification of the *IGF2* gene not only increases the expression of

IGF2, but also that of *miRNA-483*, as the latter is located on an intron of the *IGF2* gene. The amplification and overexpression of *miRNA-483* induce additional *IGF2* expression, causing a further increase in *IGF2* mRNA levels beyond those expected solely due to the amplification of the gene [129].

4.3 IGF2 Binding Proteins in Cancer

IGFBPs and IGF2BPs help in regulating IGF2 activity, bioavailability, and expression. The role of IGFBPs in cancers is not yet completely understood. Different sources demonstrate different effects of IGFBPs on cancer cells. For instance, high levels of IGFBP2 in prostate cancer were associated with low-grade cancer while IGFBP3 expression in breast cancer cells has the ability to induce apoptosis, thus suggesting an inhibitory role in IGF signaling [130]. However, other studies have shown that overexpression of IGFBP3 increased cancer growth via suppression of oxidative stress [131]. A review article by Brahmkhatri et al. suggested that IGFBPs induce apoptotic effects and inhibition of IGF1 and IGF2 signaling in cancer patients [130]. Nonetheless, literature suggests that the difference between the effect of IGFBPs on cancer lies in undiscovered factors through which, IGFBP can either elicit anti-cancer or pro-cancer effects that might be tissue and tumor subtype dependent, just similar to the different subtypes of breast cancer cases discussed earlier [132, 133].

In contrast, the function of the IGF2BP family of proteins is better understood in cancer. It has been shown that IGF2BP protein expression increases IGF2 expression and that IGF2BP1 and IGF2BP3 are frequently overexpressed during cancer [81, 134, 135]. IGF2BP can not only induce proliferation and survival through the expression of IGF2 but can also upregulate different proteins, like BCRP, which may induce chemotherapy resistance in breast cancer cell lines [136]. Additionally, IGF2BP2 expression is associated with activation of MAPK pathway by the protection of *RAF1* mRNA from degradation in colon cancer [137] as well as a variety of other pathways [82]. Also, *IGF2BP2* overexpression interrupts the differentiation of cancer cells by the *Let-7* miRNA and thus maintains the self-renewing properties of cancer stem cells in glioblastoma [138]. Similarly, up-regulated IGF2BP expression was found to be associated with poor prognosis in some cancer types [139, 140]. Additionally, IGF2BPs are over-expressed in cancer stem cells, which can initiate tumor growth and metastasis. IGF2BP1 has been shown to be up-regulated in tumor-initiating cells during colorectal cancer [83] while IGF2BP3 in cancer stem cells during HCC [141]. IGF2BP3 expression not only compliments tumor growth but also intensifies invasion in these cells, thereby contributing to metastasis. However, this effect is only partially mediated by the stabilizing effect exerted by IGF2BP3 on *IGF2* mRNA. The stabilization of *CD44* mRNA, which encodes a cell adhesion protein involved in proliferation, differentiation, and migration, shows to contribute more to the malignant phenotype of cancer stem cells than the stabilization of IGF2 [84]. Furthermore, the main role of IGF2BP in IGF2 signaling appears to be stabilization of *IGF2* mRNA, thus increasing IGF2 bioavailability. However, in regards to cancer, the stabilizing function exerted by IGF2BP in the IGF2 pathway appears only to be a small part of the total malignant effect that IGF2BP can exert through other pathways.

5. IGF2 Targeting in Cancer and Therapy Resistance

The overall poor prognosis of patients with overexpression of IGF2 or IGF1R proteins is not the only result of stimulated proliferation by IGF2 and IGF1R. Increased IGF1R signaling can cause resistance to chemotherapeutic agents through inhibition of apoptotic signaling [142, 143]. Likewise, IGF2 over-expression and activity have been linked to chemotherapy resistance and may, therefore, contribute to overall poor survival in patients with overexpression of IGF2 [144, 145]. Target compounds for receptors that bind IGF2, like the IGF1R, have been tested in several cancers. In colorectal cancer, the overproduction of IGF2 drives a subset of cancer cells that respond to IGF2 antibody treatment. The antibody, MEDI-573, neutralizes IGF2 and causes apoptosis, thus inhibiting tumor growth in mice with colorectal cancer [146]. Furthermore, similar results were obtained on anti-IGF2 antibody treatment of mice with rhabdomyosarcoma. Mice treated with the antibody were partly protected from metastasis, whereas this was not the case when the mice were inoculated in a non-IGF2-dependent salivary gland tumor [43]. In triple-negative breast cancers that express high amounts of IGF2, compounds inhibiting IGF1R exhibited a significant decrease in cell proliferation and a significant increase in cell death [147]. Several clinical trials targeting IGF1R with inhibiting compounds or IGF1R ligand antibodies, in combination with chemotherapeutic agents or as monotherapy have been tested. However, it was observed that these compounds did not effectively reduce tumor size or increased overall survival, nor did they re-sensitize tumors to the chemotherapeutic agent [141]. Clinical trials have shown limited increase in progression-free survival and overall survival in pancreatic cancer patients, while other cancers were observed to be unaffected even by combination therapy with chemotherapy and IGF1R monoclonal antibodies [141]. Yet, therapies specifically targeted at IGF2 signaling are still being considered in several types of cancers. Decreasing IGF2 bioavailability may reduce the oncogenic effects of IGF2 in cancer patients. One approach for decreasing IGF2 bioavailability is the bivalent binding of synthetic compounds which binds both IGF2 and IGF2R. In virtue of this binding, IGF2 can be internalized by the IGF2R and degraded in the lysosome. These compounds have been found to be successful at reducing cell viability, IGF2 induced proliferation, and survival *in vitro* [148]. Another channel for reducing IGF2 bioavailability would be to disrupt the imprinting of *IGF2* gene in such a way that neither paternal nor maternal *IGF2* copy expresses *IGF2*. In mice, it has been shown that 5-azacytidine, which inhibits DNA methylation, causes hypomethylation of the ICR in the *IGF2* gene (Figure 3). Since the maternal ICR in *IGF2* gene is not methylated and as a consequence of 5-azacytidine treatment, ICR on the paternal *IGF2* becomes hypomethylated and *IGF2* expression from the paternal gene would be strongly inhibited [149]. In line with this, 5-azacytidine treatment inhibited rhabdomyosarcoma cell growth through repression of *IGF2* expression and re-expression of *H19 in vitro* [150]. Additionally, it has been shown that IGF2 can maintain cancer stem cell populations in breast cancer [151] and HCC [152]. However, maintenance of cancer stem cell population seems to be more dependent on IGF1R, the previously discussed healthy stem cells signaling primarily through IRA [153]. In HCC, IGF1R inhibition with NVP-AEW541 has been found to reduce the self-renewal ability of stem cells *in vitro* [152], while stem cells for breast cancer were primarily found to express IGF1R instead of IRA [151]. Furthermore, IGF2 signaling inhibition may also be combined with other types of therapies. As outlined earlier, the anti-IGF2 antibody, MEDI-573 has shown anti-cancer efficacy in colorectal cancer in mice. However, the same study showed that combining MEDI-573 with other targeted

therapies like trastuzumab or selumetinib increased the efficacy of MEDI-573 and decreased tumor volume *in vivo* [146]. Furthermore, IGF2 expression has been shown to be relevant when cancers are treated with HDAC inhibitors. HDAC inhibitors are a rather recently developed class of compounds that inhibit the deacetylation of DNA and thus affect gene expression in cells. In cutaneous T-cell lymphomas like Sezary syndrome and mycosis fungoides, HDAC inhibitors have shown higher treatment potency than conventional chemotherapy in addition to less severe side effects as compared to the chemotherapeutics [154]. However, in solid tumors, the efficacy of HDAC inhibitors is generally limited. A phase 2 trial in non-small cell lung cancer (NSCLC), the HDAC inhibitor vorinostat showed only limited improvement in progression-free survival and overall survival [155]. The limited efficacy of HDAC inhibitors in NSCLC could be explained by the resistance caused by IGF1R-induced signaling, as IGF1R inhibition re-sensitized tumor cells to HDAC inhibition *in vitro* [155]. Furthermore, HDAC inhibition with vorinostat induced *IGF2* transcription in NSCLC through binding of STAT3 to the P3 and P4 promoters in *IGF2* gene, which subsequently led to the activation of IGF1R pathway [156]. Therefore, the activation of *IGF2* transcription and subsequent activation of IGF1R signaling by HDAC inhibitors can explain the resistance of tumors like NSCLC to HDAC inhibition. Yet, further research is warranted to discover whether combining HDAC inhibition with therapies that inhibit IGF2 or STAT3 signaling could possibly overcome the resistance to HDAC therapies by limiting the HDAC induced IGF2 signaling.

Most research in IGF2 targeted cancer therapy has been focused on IGF1R mediated chemotherapy resistance. However, as cancers preferentially express the mitogenic IRA variant of IR and since IGF2 binds with a higher affinity to IRA than to IGF1R [96], IGF2 signaling through IRA could also mediate chemotherapy resistance through mitogenic IRA signaling. Yet, this area of research has not been explored until now. In general, the role of IRA has been studied infrequently as compared to IGF1R, in cancers. Apart from combination therapies, signal inhibition of IGF1R has been extensively tested in clinical trials. However, monotherapy with IGF1R inhibitors has exhibited only limited anti-cancer efficacy in many cancers in-spite of using multiple compounds [141]. Considering that IGF1R and IRA stimulate the same downstream pathway, blocking of IGF1R might not be sufficient enough to inhibit downstream signaling, which would result in limited efficacy of IGF1R targeted therapies. A study established that in Ewing sarcoma, IGF2 signaling through IRA was able to mediate resistance to IGF1R targeted compounds *in vitro* [157]. Furthermore, disruption of IGF1R signaling increased tumor aggressiveness via increased insulin sensitivity and an increased ratio of IRA:IRB receptors present on the cell surface in an MMTV-Wnt1 mouse tumor model [158]. This further illustrates the possibility of dual blocking of the IGF1R and IRA as a potential cancer treatment. Additionally, it could also indicate that IGF1R targeted treatments may exert a higher anti-cancer efficacy in tumors that only express IGF1 and not IGF2, as IGF1 does not bind to IRA as efficiently as IGF2.

However, signaling via systemic insulin and IGF2 not secreted by the tumor could again induce IRA signaling and thus resistance to IGF1R inhibition. Therefore, for a more efficient IGF1R treatment, a combined approach with targeted therapy including IRA inhibitors or IGF2 neutralizing antibodies, and their further advancement would serve as a potentially attractive option to induce anticancer efficacy.

6. Conclusion

IGF2 is mainly expressed during prenatal life where it plays a role in the overall growth and development of the fetus. After birth, IGF2 expression decreases and might function as a metabolic factor, although the metabolic effects of IGF2 have not been completely revealed, even though it is significant for placental function.

In cancer, IGF2 signals as a mitogenic peptide having an ability to induce tumor cell proliferation and survival, thereby leading to poor prognosis in many cancers. Although the downstream signaling of IGF2 during cancers is well understood, the targeting of IGF2 related signaling remains ineffectual, partly due to lack of knowledge about IGF2 regulation and the functional proteins involved in IGF2 signaling like IGFBPs, along with other determinants of transcription. At first, the imprinting of *IGF2* and *IGF2* overexpression due to LOI is well described, although LOI is not enough to explain the level of overexpression of IGF2 in all tumors. Discovering the factors involved in the upregulation of *IGF2* expression might provide new treatment targets, like the aforementioned ACL1 or ID1, during IGF2 signaling pathway in different cancers or STAT3 as seen during HDAC inhibition treatment. In the second place, since IGF1R targeted therapies have demonstrated only limited clinical efficacy, it seems that blocking IGF2 signaling solely, would not be sufficient for treating cancers. Therefore, further research must be carried out to study the effects of blocking out all IGF-related signaling in cancer cells, including insulin, IGF1, and IGF2 signaling through the main receptors, IGF1R and IRA. Next, IGF2BPs may be a novel treatment target, not only due to the upregulation of IGF2 by IGF2BPs but also due to induced chemotherapy resistance by IGF2BPs, thus rendering inhibition of IGF2BPs a potential treatment option either in the form of monotherapy or combination therapy with chemotherapeutics. Finally, the function of IGFBPs must be studied further to ascertain from the conflicting reports, whether IGFBPs stimulates or inhibits the development of cancers. Much like TGFβ, it seems that IGFBPs can either stimulate or inhibit cancer growth depending on the tumor, receptor expression, and tissue environment. In essence, IGFBPs targeted therapies could be considered in tumors where IGFBPs induce proliferation and survival of cancer cells. Overall, IGF2 is a strongly mitogenic peptide related to poor prognosis in cancer patients and should, therefore, be subjected to further research as a potential treatment target for anti-cancer therapies. However, the function of the IGF2 protein itself is by and large well-understood in cancer. Therefore, future research should be aimed at determining the factors and proteins which affect IGF2 signaling and potency, mainly involving the function of IGFBPs, IGF2BPs and transcription factors all which can affect the bioavailability of IGF2. The functions of the different IGFBPs, especially in relation to IGF2 signaling, need to be better understood in cancer to shed light on why IGF2 inhibition may have lower anti-cancer efficacy, in certain. Furthermore, IGF2 signaling induced by several anti-cancer agents like chemotherapy and HDAC inhibitors causes drug resistance in several cancers. Testing of IGF2 blood concentration during treatment with these drugs could possibly predict the response. If IGF2 blood concentration of patients rises during treatment, IGF2 might be a biomarker for the possible effectuality of the treatment. In addition, additional drug combinations of IGF2 pathway inhibitors with drugs that induce IGF2 expression in cancers should be considered as a potential treatment for the same, so that even in case of IGF2 induction by anti-cancer drugs, IGF2 does not induce drug resistance.

Author Contributions

BTR wrote the manuscript. BTR and KS conceived the ideas of the identified problems. KS made suggestions regarding the general outline of the article and additional discussion topics.

Competing Interests

The authors have declared that no competing interests exist.

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