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Review

The Role of Insulin-like Growth Factor-1 Signaling Pathways in Uterine Leiomyoma

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Abstract. *A growing body of evidence suggests the association of the IGF-I bio-regulatory system with leiomyoma occurrence and growth. The complex interplay between IGF-I/IGF-IR and hormonal and other growth factors is, thus, now receiving significant attention. Elucidation of the molecular mechanisms driving the disease may allow for development of novel targeted-therapeutic strategies for the treatment of leiomyomas. Herein, we provide a concise update and overview of the function and regulation of IGF-I and its role in leiomyoma growth.*

Uterine leiomyomas, also known as myomas and fibroids, are benign monoclonal tumors of smooth muscle cells. They are the most common neoplasm and a major cause of morbidity in women of reproductive age (1). Leiomyomas are classified according to their location as subserosal, intramural, or submucous. They are formed by overgrown smooth muscle cells which express high extracellular matrix proteins such as collagen, fibronectin and proteoglycans. Several epidemiological and cytogenetic studies have suggested that a genetic component is playing a role in the pathogenesis and progression of the disease (2-5). Ethnicity, lifestyle, early menarche, parity and pregnancy, caffeine intake, dietary, metabolic and environmental factors, diseases such as diabetes mellitus and polycystic ovaries have been associated with the occurrence of leiomyomas (6). Since leiomyomas rarely appear before menarche and almost always regress after menopause, the role of sex steroids and other growth-related or growth factors have been implicated in the pathophysiology of the disease (7-11). Herein we review the evidence implicating the insulin-like growth factor I (IGF-I) bioregulation system in the pathophysiology of uterine leiomyomas.

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The IGF-I Bioregulation System

The IGF system is a complex biological system comprised of peptide hormones, *i.e.*, the insulin-like growth factor-1 and -2 (IGF-I and IGF-2) and insulin; cell surface receptors, *i.e.*, the IGF-1 receptor (IGF-1R), insulin receptor (IR), and hybrid IGF-1R/IR; as well as IGF binding proteins (IGFBPs) which regulate a number of crucial biological processes, including cell proliferation, differentiation, migration and survival of smooth muscle cells (12-14) (Figure 1).

IGF Binding Proteins (IGFBPs)

The biological actions of IGFs are modulated by a family of six important IGFBPs (IGFBP-1 to -6) that have affinity for IGF-1 and IGF-2 and control the local bioavailability of IGF-1 and IGF-2 (14-19). IGFBs also increase the half-life of circulating IGFs that are protected from proteolytic degradation by forming a ternary complex with IGFBP-3 and the glycoprotein acid-labile subunit (ALS) (20, 21). Compared to IGF-IR, IGFBPs have a higher binding affinity for IGFs. By controlling the local tissue bioavailability of IGFs, IGFBPs regulate the tissue specificity of IGFs (15, 21-26). Of note, certain IGFBPs can exhibit IGF-potentiating effects. Various factors, such as the tissue-specific distribution

of particular IGFBPs and the ratio between free (active) IGFs and bound IGFBP-IGFs complexes, influence whether IGFBPs stimulate or inhibit IGF activity (27, 28). Moreover, it has been shown that certain IGFBPs have IGF-independent activities indicating that they can modulate cell apoptosis and survival, or inhibit tumor growth in the absence of the ligand (26, 28, 29). In addition, proteolytic fragments of IGFBP-3 have demonstrated IGF-independent mitogenic activity in the peritoneal fluid of women with endometriosis (30).

IGF Receptors

IGF activity is mediated through binding to several receptors, including the type 1 (IGF-1R) and type 2 (IGF-2R) IGF receptor, the insulin receptor (IR), and some atypical receptors such as the hybrid IR/IGF-1R (31-33). IGF-1R binds IGF-1 with higher affinity than IGF-2 and insulin. IGF-2R binds IGF2 with much higher affinity compared to IGF-1, and does not bind insulin. The IR/IGF1R hybrid receptor binds both insulin and IGF-I; nevertheless it is thought to function predominantly as an IGF-1R as its binding affinity for insulin is much lower than its affinity for IGF-I. The functional importance of the hybrid IGF-1R/IR receptor remains to be defined (Figure 1) (19, 21, 34, 35).
Given the significant structural similarity between IGF-1 and insulin, and the high degree of homology that IGF-1R exhibits to IR (36), these ligands can cross-activate both receptors, while the IGF-1R signaling pathways share multiple intracellular mediators with the insulin signaling cascade (17, 19). Nevertheless, IGF-I, IGF-II and insulin can also produce unique signaling outcomes (14).

IGF-1

IGF-1 is a secreted growth factor, critical for normal body growth, development and maintenance and plays important roles in multiple biological systems (26, 35, 37, 38). Unlike other growth factors, IGF-1 acts as both a mitogen and a differentiation factor (39) and it has been implicated in various conditions, including several cancers (28, 40, 41), as well as the myogenic processes during muscle development and regeneration (42). IGF-1 can act as both an endocrine and an autocrine/paracrine factor. As a circulating hormone, IGF-1 mediates the effects of pituitary growth hormone (GH) (36, 43, 44). Circulating IGF-1 is mainly derived from the liver but also from skeletal muscles (38, 45-47) and it is mostly bound to IGFBPs (35, 48).

The human *Igf1* gene contains six exons that give rise to various mRNA transcripts by a combination of alternative 5'-leader sequences and 3'-splicing (49-51) (Figure 2). Specifically, the different leader sequences result in two different classes of *IGF-1* mRNA variants. Class 1 transcripts have their initiation sites on exon 1 (promoter 1), whereas class 2 transcripts use exon 2 as the leader exon (promoter 2), producing class 1 or class 2 transcripts by differential splicing of exons 1 or 2 to the common exon 3. Alternative splicing of exon 5 also results in different mRNA variants containing exon 6 and excluding exon 5 (IGF-1Ea) or containing exon 5 without exon 6 (IGF-1Eb) (26, 52-54). A third variant, the IGF-1Ec, is also generated by alternative splicing in the human *Igf1* gene and contains parts of both exon 5 (49 bp) and 6 (55). All possible combinations between leader sequence (exon 1 or 2) and terminal exon (5 or 6) can occur in different IGF-1 transcripts (Figure 2) (19, 52, 56). Transcripts initiating at promoter 1 are widely expressed in many tissues, whereas transcripts initiating at promoter 2 are expressed mainly in the liver and kidney (57) and can be GH-dependent (51, 58-62), or GH-responsive (63, 64). However, the two promoters are likely not mutually exclusive and GH can also stimulate the expression of tissue-specific transcripts, although current evidence of this remains equivocal (Figure 2) (65-70).

Recent studies in humans have shown that the IGF-I splice variants are differentially transcribed in response to various conditions and pathologies (26), such as exercise-induced muscle damage (71, 72), endometriosis (73), prostate (74), and cervical or colorectal cancer (75, 76), as well as in some human cell lines following hormonal treatment (70, 77, 78). The differential expression of IGF-1 transcript variants in various pathologies could indicate distinct regulatory mechanisms and diverse responses of cells to different stimuli (21), and may reflect IGF-1 isoform-specific biological roles in different conditions (65). However, the specific functions of different IGF-1 splice variants remain largely unknown.

The different *IGF-1* mRNA transcripts encode the corresponding precursor proteins IGF-1Ea, IGF-1Eb and IGF-1Ec (26, 51). The IGF-1 protein isoforms share the same mature peptide, which is the common part of all the IGF-I precursors (51, 55, 79, 80). Post-translational conversion of pro-IGF-1 polypeptides to mature peptide cleaves off their E domains (E peptides) and three different E peptides have been identified in humans, namely the Ea, Eb and Ec peptide (Figures 2 and 3) (19, 26).

Bioactivity of IGF-1 Peptides

Although IGF-1 activity is mediated by several receptors, most of its biological effects on cell growth, differentiation, survival and invasion depends on the binding to IGF-1R, which is a ligand-activated receptor tyrosine kinase (27, 81). Trans-autophosphorylation of the cytoplasmic tyrosine kinase domain of the receptor leads to the recruitment of specific cytoplasmic molecules and activation of specific intracellular pathways including Ras/mitogen-activated protein kinase (MAPK)/ERK1/2 and phos-phatidylinositol 3-kinase (PI3K)/Akt (Figure 3) (26, 72, 82-84).

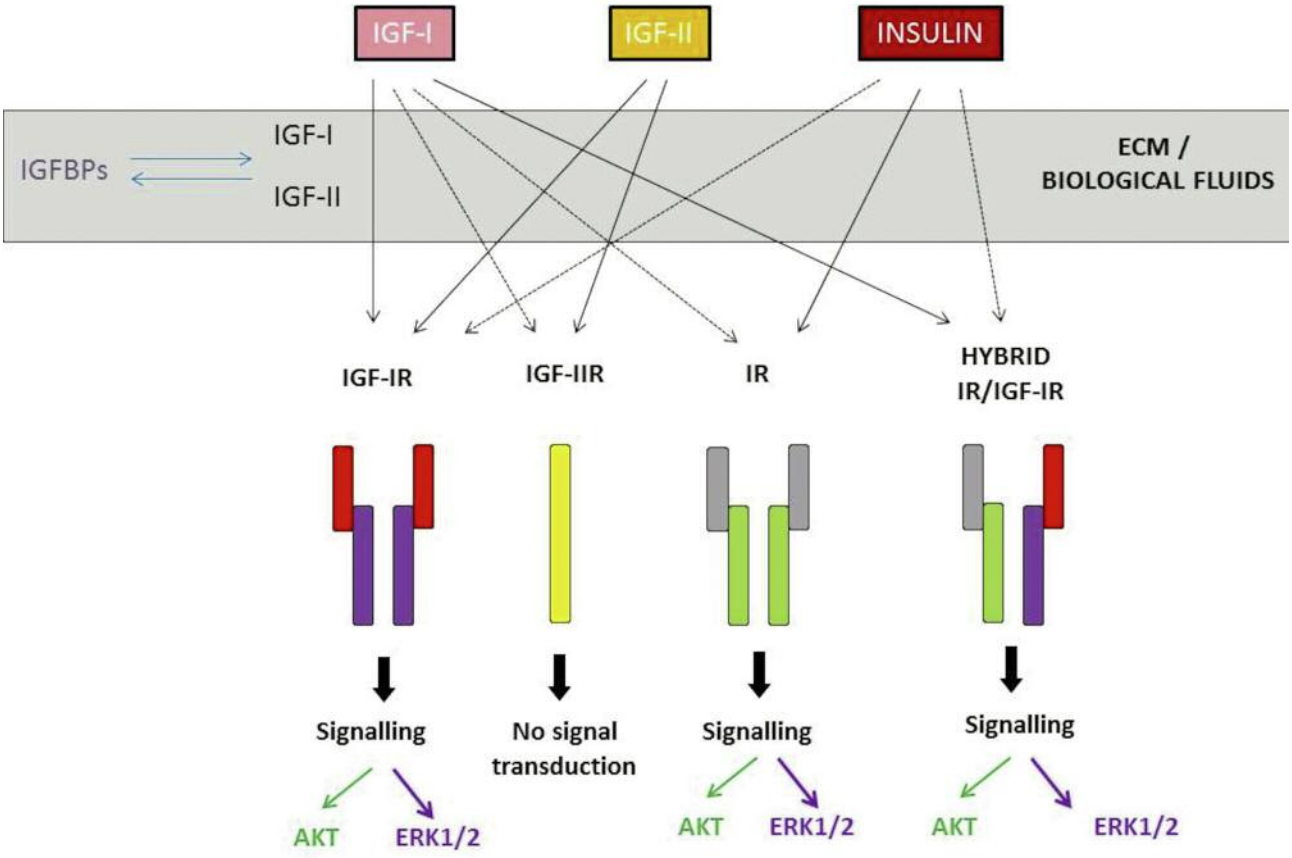

Figure 1. *The IGF bio-regulation system consists of IGF-I, IGF-II, insulin, the type I (IGF-IR) and type II (IGF-IIR) IGF receptors, insulin receptor (IR) and IR/IGF-IR hybrid receptors, as well as at least six high-affinity IGF binding proteins (IGFBPs). IGFBPs increase the half-life of IGFs in the extracellular matrix (ECM) and modulate the biological actions of IGF. The IGF ligands exhibit differential binding affinity to the IGF receptors (solid arrows indicate a higher binding affinity compared with the dashed arrows) and share multiple signaling pathways and intracellular mediators, including ERK1/2 and Akt. The IGF-IIR actually does not have an intrinsic signaling capability and it primarily internalises and degrades IGF-II, sequestering it from potential receptor activating interactions.*

By general consensus, the IGF-1 domain that is responsible for receptor binding is considered to be the mature IGF-1 peptide. However, differential biological activities have been reported for the different IGF-1 isoforms (pro-peptides), or for their E peptides, overexpressed or exogenously administrated in various *in vivo* and *in vitro* models (26), and it was suggested that there are peptides, other than the IGF-1 ligand, that also possess bioactivity (79, 85, 86). This concept was further supported by findings that revealed differential E peptide- or IGF-1 isoform-specific signaling (72, 74, 86-90).

Synthetic E peptide analogs generated from unique regions within the E domains have been shown to possess *in vitro* mitogenic, angiogenic and migratory activities, and regulate cell differentiation in various human cells or cell lines (26). Similarly, studies using animal models have shown that exogenous administration or over-expression of synthetic peptides, generated particularly from regions within the human Ec peptide, produces unique though inconsistent effects in cell proliferation and migration, and can delay or inhibit cell differentiation (91). The differential biological effects of the synthetic Ec peptide compared to mature IGF-1 peptide and the lack of suppression of synthetic E-peptide bioactivity after blocking mature IGF-1 signaling with IGF-1R neutralizing antibodies suggest that the Ec peptide may act *via* a different receptor (92-94). Evidence for a distinct, independent of IGF-1R, bioactivity of the human Ec domain, was also provided by its divergent signaling compared to mature IGF-1. Specifically, *in vitro* studies have shown that a synthetic analog of the human Ec peptide activates distinct signaling pathways compared to the IGF-IR ligand, since in contrast to mature IGF-1, it only activates ERK1/2 and not Akt (Figure 3) (70, 72, 74, 86-88). Moreover, the IGF-1R- and IR-independent bioactivity of this synthetic part of the Ec domain and its selective activation of only one of the two main signaling pathways downstream of

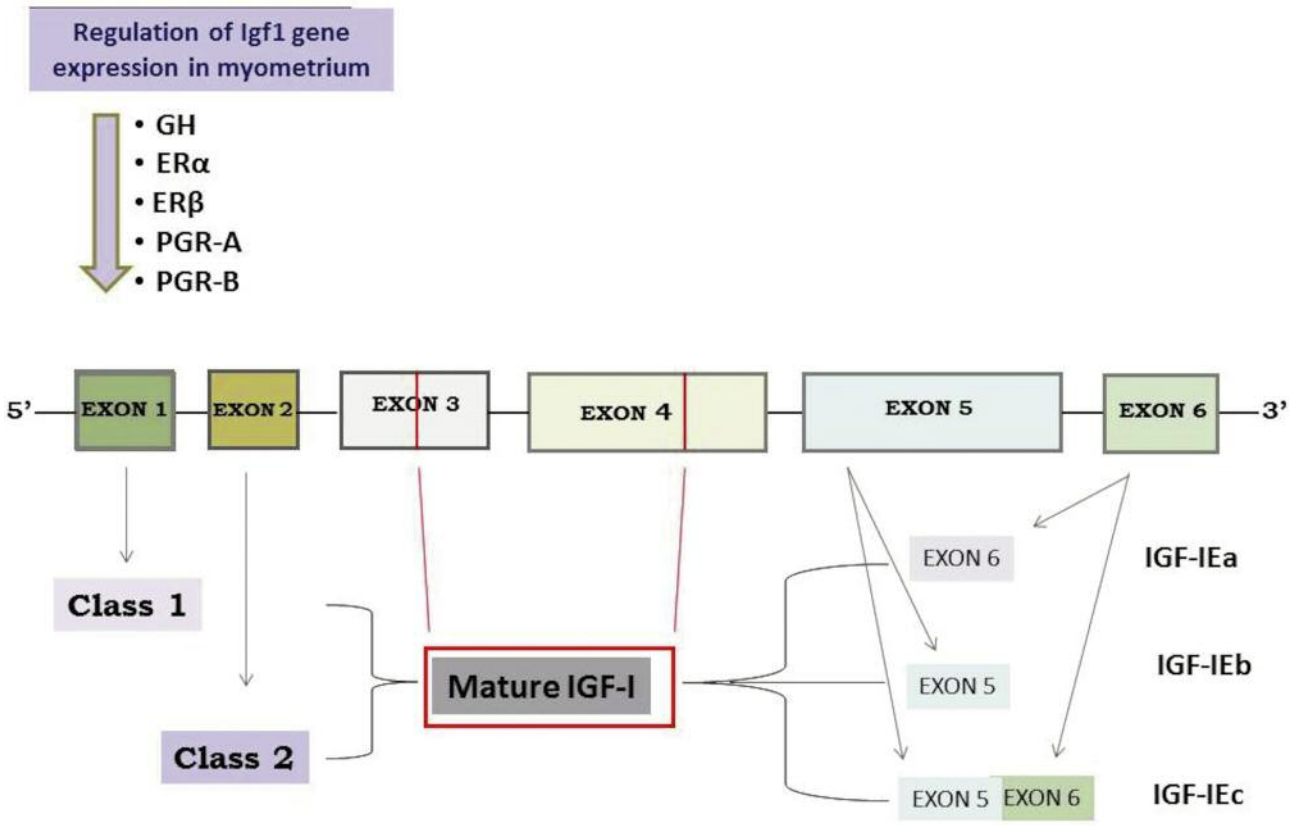

Figure 2. *Schematic representation of the human Igf1 gene structure and alternative splicing. The Igf1 gene contains six exons and its different leader sequences result in two different classes of mRNA variants. Class 1 transcripts have their initiation sites on exon 1, whereas class 2 transcripts use exon 2 as the leader exon. Alternative splicing of exon 5 results in different mRNA variants containing exon 5 (IGF-IEb), or exon 6 excluding exon 5 (IGF-IEa), or containing parts of both exon 5 and 6 (IGF-IEc). The mature IGF-I peptide, which interacts with IGF receptors and binding proteins, is coded by parts of exons 3 and 4. Potential regulators of IGF-I expression and alternative splicing in myometrium are shown. GH: Growth hormone; ERα: estrogen receptor-a; ERβ: estrogen receptor-β; PGR-A: progesterone receptor-A; PGR-B: progesterone receptor-B.*

IGF-1/IGF-1R were further supported by siRNA knock-out experiments in various human cell lines (73, 74, 77).

More recently, synthetic E peptides corresponding to the rodent Ea and Eb domain sequences were used in a mouse *in vitro* model to evaluate the IGF-1-dependent and -independent activation of IGF-1R by those E peptides. The results of these studies suggested that E peptide signaling, as well as its mitogenic effects, are dependent upon a functional IGF-1R, and act as part of pro-IGF-1 (26, 90, 95). Further, evidence supporting the bioactivity of pro-IGF-I forms has been provided in a murine model (96). It should be noted, however, that concerns have recently been raised regarding the existence of any physiological role for a secreted rodent Eb or human Ec peptide (97). However, it has been suggested that species specificity must be taken into account when assessing the activity of the human IGF-1 Eb and Ec domains, from which peptides with important biological activities have been reported, since the IGF-1 E domains are very variable and much less conserved among species compared to the other IGF-I domains (26, 51). Thus, it remains to be elucidated whether the autonomous, IGF-1R- and IR-independent bioactivity of human Eb and Ec peptides reported in various human cell lines (19, 73, 74, 79, 98), reflects an alternative, species-specific ligand/receptor mechanism of action for these human E domains (26).

Moreover, it is still not known where the Ec peptide signaling diverges from that of mature IGF-1 (given the distinct activation of ERK1/2, but not Akt pathway by the Ec peptide), (Figure 3). It remains to be elucidated whether the Ec peptide affects the ERK1/2 pathway at the level of the IGF-1 receptor(s), or this activation occurs at a level downstream of the IGF-1R, *via* an intracrine signaling mechanism, or the Ec peptide utilizes a cellular uptake mechanism or a separate receptor that can activate ERK1/2. However, the existence of such a putative, canonical or non-canonical receptor or internalization mechanism for the Ec peptide remains to be determined and characterized (26).

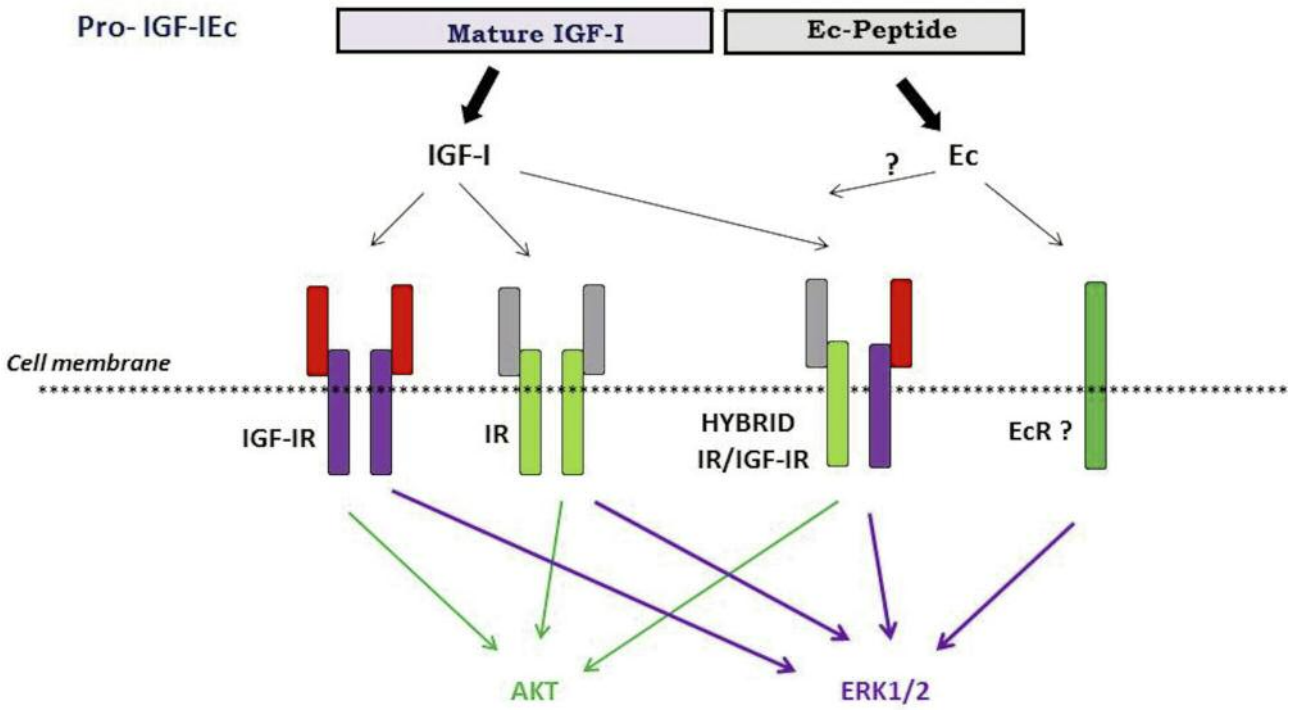

Figure 3. *Mature IGF-I-induced activation of Akt and ERK1/2 signaling proteins via IGF receptors. IGF receptor-independent signaling of the Ec peptide, mediated by a putative Ec receptor (EcR) that would preferentially activate ERK1/2, is postulated.*

The Role of IGF-1 System in the Pathogenesis of Uterine Leiomyomas

In vivo and *in vitro* studies have suggested that IGF-1 is an important factor in the growth process of fibroids. Both myometrium and leiomyoma tissues in humans contain large amounts of extractable IGF-I, much higher compared with other peptide growth factors (99). The stimulatory effect of IGF-1 in leiomyoma growth *in vitro*, in the absence of steroid hormones, indicates the direct role IGF-1 in the pathogenesis of leiomyoma. On the other hand, molecular pathogenesis of fibroid growth depends on sex steroid hormones, which mediate fibroid growth by binding to their receptors, with subsequent activation of proto-oncogenes, growth factors and their receptors (100). Clinical observations provide strong evidence that leiomyoma is estrogen- and progesterone-responsive. The disease incidence of fibroids increases in association with high estrogen and progesterone levels, particularly during the reproductive years, and decreases after menopause. This hormonal influence is evident in the increase in fibroid size seen in menopausal women taking estrogen and progesterone replacement therapy (101, 102).

Several studies have assessed *IGF-1* mRNA expression in fibroids and myometrium. Englund *et al*. (103) reported that there is no difference in the *IGF-I* mRNA expression between fibroids and matched myometrium, whereas there is a significantly higher level of *IGF-1* mRNA expression in the follicular phase than in the luteal phase of the menstrual cycle. They also reported a 4-fold difference in mRNA expression between fibroids from the same patient, a fact that indicates the importance of examining more than one fibroid from each patient. In the case of gonadotropin-releasing hormone (GnRH) agonist-treated women and post-menopausal women, the reduction of fibroid and uterine volume is not only associated with decreased hormonal concentrations but also with decreased levels of *IGF-1* mRNA expression. The authors however did not find any significant difference in IGF-1 peptide expression, suggesting a post-transcriptional modification of the *IGF-1* gene expression. Lower IGF-1 levels in explant cultures of leiomyomas taken from patients treated with GnRH–analogs compared to those taken from untreated patients have been reported (104). Peng *et al*. (105) observed higher *IGF-1* mRNA levels in the proliferative compared to the secretory phase, both in fibroids and matched myometrium, in premenopausal women. The effect of estrogen on mRNA expression was similar in both tissues and there was no association with tumor size or age. A differential expression of IGF-I at the protein level was apparent in fibroids of larger size (105).

Over-expression of the IGF-1 peptide has been reported in fibroids of humans and Eker rats (103, 106, 107). IGF-

binding proteins IGFBP-2, -3 and -4 have been detected in the media of leiomyoma and myometrium explant cultures (108-114). The endocrine/paracrine/autocrine activation of IGF-IR can be accomplished by IGF-1 and IGF-2 and plays a very important role in the growth of normal human myometrium, thus conceivably affecting the growth of uterine leiomyomas from myometrium (105, 108-111). IGF-IR has been detected in the uterus (myometrium, epithelium, and stroma) (115) and its levels are significantly higher in leiomyoma than in myometrium cell membranes (116, 117). IGF-1 acts as a survival factor that inhibits apoptosis in a variety of cell types, thus over-expression of IGF-1R in cells increases their tumorigenic potential and protects them from programmed cell death (118). IGF-1R can play a tumorigenic role *via* the hyper-activation of IGF-1 signaling induced by the over-expression of IGF-1R, over-abundance of the ligand, or up-regulation of the PI-3K/Akt pathway (106). Phosphorylated IGF-1R, as well as Shc, and ERK1/2 are over-expressed in IGF-1-treated uterine leiomyoma (UtLM) cells, and this effect can be blocked by a neutralizing antibody against the IGF-1R(119). Constitutively activated ERK 1/2 is highly detected in leiomyoma and myometrial tissues. Moreover, *in vitro* microarray analyses have shown that IGF-I and other novel genes, potentially involved in the IGF-1R-MAPK signaling pathway, are differentially expressed in uterine leiomyoma cells compared to uterine myometrial cells following estradiol (E2) treatment (119). Furthermore, in order to identify whether receptor tyrosine kinase (RTK) pathways are involved in estrogen-regulated uterine leiomyoma growth, the levels of estrogen receptor (ER)α phosphorylated at Ser118 (ERa-phospho-Ser118) and phosphorylated MAPK (phospho-MAPK) in tumors during the proliferative phase were investigated (119). Co-localization of ERα-phospho-Ser118 and phospho-MAPK were more concentrated in the nuclei of leiomyoma cells compared with myometrial cells, while increased immunoprecipitation of ERα-phospho-Ser118 and phospho-MAPK was also observed in leiomyomas compared with myometrial tissue during the proliferative phase (119). Di Lieto *et al*. (120) investigated the pharmacological action of GnRH analogs on uterine leiomyomas and found that, in addition to causing uterine volume reduction, there was a reduction in IGF-R levels likely due to the hypoestrogenic state induced by the treatment.

The effect of IGF-I in leiomyoma cells in tissue culture is dose-dependent (121). IGF-1 signaling consists of many intracellular pathways such as the Ras/Raf/MAPK and the PI3K pathways (122). The tyrosine kinase receptor IGF-IR uses IRS-I/Shc as an intermediate for the activation of the IRS/PI3K/Akt pathway for cell survival as well as the Shc/Ras/Grb2/MAPK pathway for cell proliferation. Leiomyomas exhibit elevated phosphorylation of MAPK compared to the myometrium (123). The PI3K-Akt-mTOR pathway plays an important role in many cell functions, such as growth, survival, and proliferation. The activation of PI3K is mediated either by RTKs or *via* G-protein coupled receptors coupled, oncogenes or steroid hormones. RTKs are the intermediates of a transduction pathway that transmits extracellular signals within the cell and controls cell proliferation (124, 125). These receptors are considered targets for many anticancer therapies since their overexpression results in continuous RTK signaling which induces cell cycle deregulation and tumor progression (124, 126). Studies have shown that RTKs are overexpressed in leiomyomas and the myometrium. The levels of p-Akt and the downstream effectors, p-GSK3b and p-FOXO1, are increased under the effect of progestin on uterine leiomyoma cells. Akt phosphorylation is blocked by the progesterone receptor (PGR) antagonist RU 486 and the PI3K suppressor LY290004 indicating the dependence of those cells to PGR and PI3K activation. Progesterone decreases the mRNA expression of IGF-1 but at the same time IGF-1R levels remain constant (127, 128). PI3K activates AKR which regulates many functions *via* the phosphorylation of proteins such as the tumor suppressor protein tuberin (tuberous sclerosis complex 1, TSC1), and the mammalian target of rapamycin (mTOR) pathway. mTOR regulates protein translation through phosphorylation of the S6 kinase 1 (S6K1) and the elongation-initiation factor 4E-binding protein 1 (4EBP1) (127). Continuous activation of mTOR has been found in leiomyosarcomas (126). In the Eker rat model, deletion of the *tuberin* gene is associated with high incidence of uterine leiomyomas. Uterine leiomyoma cell lines derived from Eker rats have a mutation in the tumor suppressor gene Tsc-2, resulting in a high growth rate of leiomyomas (129-131). Microarray analysis of tuberin, hamartin and proteins of the insulin signaling pathways showed either decrease or complete loss of tuberin in uterine leiomyoma compared to the myometrium (131). In addition, IGF-1 can induce cell proliferation through up-regulation of Bcl-2 in leiomyomas (132). Progesterone increases the expression of the anti-apoptotic gene Bcl-2 while direct binding of PGR in the Bcl-2 promoter suppresses its transcription in primary uterine leiomyoma cell cultures (133, 134).

Regulation of IGF-1 and IGF-1R Expression by the Estrogen and Progesterone Receptors

The circulating levels of sex hormones are of major importance in uterine leiomyoma growth, and this is why these tumors stop growing after menopause. During pregnancy, some uterine leiomyomas increase in size due to increased circulating levels of estradiol and progesterone. However, most leiomyomas remain at the same size or even shrink during pregnancy, indicating that the responsiveness of leiomyomas to estrogen and progesterone may vary. At the same time, the

overexpression of peptide growth factors and their receptors mediates important autocrine/paracrine and intracrine effects that can facilitate leiomyoma growth (116, 135).

Estrogen and the sub-forms of ERs have important inter-relationships with PGRs that modulate biological responses. ERα expression in uterine cells is inhibited by progesterone *via* PGRs (136). The interaction between the progesterone and estrogen hormonal systems is of major importance for maintaining normal uterine function and for balancing the opposite actions of the progesterone/PGR and estrogen/ER systems. The mechanism of regulation of PGRs by estrogens still remains to be fully elucidated. *In vitro* studies suggest that hormones and growth factor interactions occur *via* ER activation of MAPK and estrogen pathways (128, 137, 138). The *IGF-1* gene is up-regulated by estrogen in cultured leiomyomas and leiomyoma primary cultures have elevated transcriptional activity in response to 17β-estradiol (E2) compared to autologous myometrial cultures (100). *In vivo* and *in vitro* studies have demonstrated the sensitivity of uterine leiomyomas to environmental estrogens as well as the increased incidence of uterine leiomyomas and adenocarcinoma later in life (139). Genistein, an estrogenic soy-derived compound of phytoestrogens, is usually consumed in the diet. Genistein stimulates the growth of UtLM cells by inducing interactions between ERα and IGF-1R. The use of ER antagonists and MAPK/ERK kinase inhibitors blocks the effects of genistein in UtLM cell (140). A cross-talk between ERα and IGF-I has been demonstrated in the murine uterus in response to genistein (141, 142). A low concentration of genistein can induce EPK1/2 activation in LM cells, but not in uterine smooth muscle cells (UtSMC) (140). Early activation of IGF-1R signaling after short-term genistein treatment may be mediated by interactions between IGF-1R and ERα. Such interactions may occur when ERα is bound with a ligand in leiomyoma cells, and can be blocked by estrogen antagonists.

In several cell types, ERα is involved in the early activation of ERK1/2 by estrogens (143-145). Shc, an early signaling intermediate of IGF-IR, is associated with ER-α-mediated rapid activation of ERK1/2. IGF-1R facilitates ER-α-mediated rapid action of E2 (145, 146). Estrogen produces mitogenic effects on leiomyoma cells by triggering the rapid and transient activation of the MAPK pathway. During the menstrual cycle phosphorylated Akt levels are higher in leiomyomata than in myometrium, whereas at menopause no differences are observed in the levels of p-Akt between the two tissue types (147, 148). The importance of the Akt pathway in the pathogenesis of leiomyomas is seen in the transgenic Eker rat model, where the TSC-2 gene mutation is a direct target of Akt. A high percentage (65%) of female Tsc2Ek/carriers develops leiomyomas (149). In the Eker rat model, IGF-1 exceeds maximal cell proliferation activity during pro-estrus, when both E2 and PG levels reach the highest concentration, indicating that the proliferative action of IGF-1 in the rat uterus is regulated by both sex steroids (150). Treatment with combined estrogens and progesterone, as well as treatment with progesterone alone decreases the expression of IGF-1 in uterine leiomyoma without, however, affecting the mRNA levels of *IGF-1R* (127, 151). Progesterone affects leiomyoma growth through two different ways: i) it up-regulates epidermal growth factor (EGF) and bcl-2 expression and down-regulates tumor necrosis factor a (TNFa) resulting in a stimulatory effect on leiomyoma growth, or ii) it down-regulates *IGF-1* mRNA and protein expression and leads to a decrease in the development of leiomyomas (127, 151-153). This could explain why uterine leiomyomas do not gain size in most cases during pregnancy, when progesterone levels are high. In uterine leiomyoma cell cultures, the MAPK pathway interacts with the estrogen system *via* the ERs (128). ERs are expressed at lower levels during the secretory phase (154-156). ERα-phospho-Ser118 is significantly higher in tumors from women in the proliferative phase of the menstrual cycle compared to those in the secretory phase. The proliferative phase is estrogen-dominant, indicating that phosphorylation may be regulated by higher concentrations of estrogen and lower concentrations of progesterone (157).

The two regulatory pathways, E2/ERα and IGF1/IGF-1R, are closely related in UtLM cells. The E2 effects can occur through both genomic and non-genomic cascades, which involve IGF-1R-induced activation of MAPK. IGF-1R and its signaling molecules play an important role in E2-mediated activation of MAPK and MAPK-related pathways in UtLM cells, as evidenced by the diminished response to E2 treatment after IGF-1R silencing (158). E2 increases IGF-1 expression that is mostly localized in the cytoplasm, but may also translocate to the nucleus, while there is a quick phosphorylation of IGF-1R, Shc and ERK1/2. The results of this study also indicated an association between IGF-1R and ERα, as well as between Shc and ERα, in UtLM cells exposed to E2. Many genes involved in the IGF-1 signaling pathway are differentially expressed in estrogen-treated UtLM cells, *IGF-1* and *A-myb* are up-regulated in estrogen-treated cells while *MKP-1, c-fos* and *myc* are down-regulated in uterine leiomyoma tissues when compared to autologous myometrium (138). Smooth muscle tumors in the Eker rat model behave like smooth muscle tumors in humans; they are benign tumors, responsive to hormones and express both ERs and PGRs(150). Much research has been performed in characterizing the differential expression of various growth factors and their receptors in leiomyoma and myometrium. Estrogens (E2), up-regulate the expression of IGF-1 in UtLM cells through the activation of ERα (138), (Figure 2) while PG stimulates the growth of uterine leiomyomas *via* the activation of PGRs (159). Therapy with PGR antagonists such as RU486 or selective progesterone receptor modulators results in leiomyoma shrinkage. Moreover, the two PGR

isoforms, PGR-A and PGR-B, are overexpressed in response to E2 administration in uterine leiomyomas (160). *IGF-1* mRNA levels negatively correlate with PGR-B levels in leiomyoma (161) (Figure 2). Since IGF-1 treatment can increase leiomyoma proliferation (121, 162, 163), it remains to be clarified how progesterone-induced regulation of IGFs contributes to leiomyoma growth. Studies support that ERα stimulates *IGF-1* mRNA expression and the ERα/ERβ ratio remains high in human uterus (160), while other studies failed to reveal any significant difference in ERα and ERβ expression between ULM and normal myometrium (164). ERα activation by E2 leads to the activation of IGF-1/IGF-1R/ERK1/2 pathway and genes which control cell proliferation and differentiation, such as MAPKs and cyclins, are up-regulated after exposure of cells to E2 in the presence of IGF-1R overexpression (158).

Conclusion

IGFs are potent mitogens found in a variety of organs including the uterus. Leiomyomas take advantage of the IGF bio-regulatory system to facilitate cell proliferation and survival. Key molecules in the IGF pathway include the IGF-1 and IGF-2 growth factors, the IGF-1R and IGF-2R receptors, as well as the IGFBPs. These molecules interact with major hormonal and other signaling cascades implicated in leiomyoma growth. Interestingly, it remains to be confirmed whether there is a different expression pattern of the IGF-1 isoforms (IGF-1Ea, IGF-1Eb, IGF-1Ec) between uterine leiomyomas and adjacent normal myometrium. Advances in understanding over the complex interplay between the IGF system and leiomyomas will allow for development of novel agents and therapeutic strategies.

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