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Activation of various downstream signaling molecules by IGFBP-3

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Abstract

Insulin-like growth factor binding protein-3 (IGFBP-3), a secretory protein, is the most abundant IGF binding protein present in human serum among all IGF binding proteins. IGFBP-3 shows decreased level of expression in cancerous cells but has been known to be present in significant amounts in normal or non-cancerous cells. IGFBP-3 can induce apoptosis in prostate cancer cells either in an IGF-dependent manner or independently of IGF binding. Although putative cell death specific Insulin-like growth factor binding protein-3 (IGFBP-3R) receptor(s) has recently been identified by which IGFBP-3 may induce its anti-tumor effects, IGFBP-3 has also been known to activate various downstream intracellular signaling molecules via a different mechanistic pathway. Stat-1 has been known to be one of the candidate molecules activated by IGFBP-3. IGFBP-3 can also inhibit Akt/IGF-1 survival pathway in MCF- 7 breast cancer cells which ultimately leads to the induction of apoptosis in these cells. All these studies clearly demonstrate that IGFBP-3 regulates cell proliferation and promotes its pro-apoptotic effects in cancer cells in two different pathways,1) sequester IGF-I to bind to IGF-I receptor to inhibit cell proliferation and induce apoptosis, 2) independent of IGF-I pathway, IGFBP-3 binds to some putative receptor and activate various downstream pro-apoptotic molecules involved in cell death.

Keywords

Apoptosis; IGFBP-3; Stat-1; IGF-I; TGF-β

1. IGFBP-3 expression and function

Insulin like growth factor binding protein-3 (IGFBP-3) is one of the six known IGF binding proteins present in human serum and is comprised of 264-amino acid mature protein, with a 27-amino acid signal peptide [1]. IGFBP-3 is expressed in a wide variety of tissues. Epidemiological studies have clearly suggested that there is an inverse relationship between IGFBP-3 levels and occurrence of cancers [2] Increased expression levels of serum IGFBP-3 results in decreased prevalence of prostate [3, 4] and colorectal [5, 6]cancers. It has also been shown that IGFBP-3 overexpression results in decreased tumor formation in

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xenografts of non-small lung cancer cells [7] and M12 human prostate cancer cells [8]. Patient samples with hepatocellular and non-small cell lung carcinoma has been shown to have decreased expression of IGFBP-3[9, 10]. Besides, it has also been reported that there is an increased level of IGFBP-3 in senescence [11]. In addition, IGFBP-3 expression level was decreased in cells immortalized with the human papillomavirus type 16 oncoprotein E7 due to proteasomal degradation [12]. Although IGFBP-3 is a secretory protein, but active protein could also be found in nucleus and cytoplasm [13]. Besides its full length form, IGFBP-3 could also be found in a N-terminal truncated form [14, 15].

IGFBP-3 has been known to play an important role in cell proliferation by inducing its anti-proliferative and pro-apoptotic effects in breast and prostate cancer cells [16, 17, 18]. It has also been shown that IGFBP-3 not only triggers growth inhibitory effects by inducing apoptosis but it can also function in G1 cell cycle arrest in human breast, kidney and lung cancer cells [19, 20]. IGFBP-3 may also play an important role in mediating inhibitory effects of transforming growth factor (TGF)-β (Figure 1), retinoic acid, tumor necrosis factor (TNF)-α and p53 [16, 21–26], but modulation in IGFBP-3 synthesis or action could regulate these anti-proliferative effects.

2. IGF-dependent and independent effect of IGFBP-3

IGF dependent studies revealed that Insulin-like growth factor binding protein (IGFBP)-3 could induce apoptosis by binding to IGF-I and form a binary complex with IGF-I and prevent it to activate (IGF-IR) IGF-1 receptor (Figure 2) to stimulate cell proliferation and survival [27, 28]. In addition, Insulin-like growth factor binding protein (IGFBP)-3 has been shown to potently inhibit cell proliferation and induce apoptosis in an insulin-like growth factor (IGF)-independent manner [13, 18, 29]. IGFBP-3 in the media may exert its pro-apoptotic effects by binding to some cell surface receptors resulting in the activation of various signal transduction pathways. Alternatively, IGFBP-3 may possibly enter into the cell by endocytosis (Figure 3). It has also been reported in some other studies that IGFBP-3 binds to RXRα (Figure 1) and induce its pro-apoptotic effects in PC-3 human prostate cancer cells [30]. Surprisingly, recent studies have shown that IGFBP-3 fail to gain entry or be internalized upon binding to IGF-1 in the extracellular media to induce its pro-apoptotic effects in non-transformed mammary epithelial cells [31].

3. Pathways involved in IGFBP-3 signaling

IGFBP-3 action results in the activation of various signal transduction pathways [32] and Stat-1 (signal transducer and activator of transcription 1) has been known to have a functional role in IGFBP-3-induced apoptosis (Figure 1) in rat chondroprogenitor cells [33], although our previous studies [34] showed a protective role of Stat-1 on IGFBP-3 induced apoptosis in PC-3 human prostate cancer cells, implying the fact that role of Stat-1 may be cell type dependent.

Some other downstream signaling molecules of IGFBP-3 have also been reported in various other studies [16, 35, 36]. It has been shown from these studies that IGFBP-3 can bind to transforming growth factor-β (TGF-β) cell surface receptors and one of the studies have

shown direct interaction of IGFBP-3 with TGFβ-receptor typeV (TGFβ-RV) in mink lung epithelial cells [37].

IGFBP-3 has also been known to bind and activate intracellular signaling by forming a hetero-meric complex with other TGF-β receptors (TGF-β RII and TGF-β RI) in T47D breast cancer cells [35, 36]. Besides, addition of IGFBP-3 resulted in the activation of Smad2 phosphorylation and cell growth inhibition in these cells. It has also been shown that IGFBP-3 mediates its pro-apoptotic effects via TGF-β in PC-3 human prostate cancer cells [16]. Results from our previous studies indicated the inhibition of TGF-β signaling in presence of IGFBP-3 in PC-3 human prostate cancer cells [34], although we did not study the Smad activation in these cells.

It has also been reported that IGFBP-3 down-regulates Akt activity in human epidermal growth factor receptor-2 (HER-2) overexpressed MCF-7 breast cancer cells [38], in this regard recent studies have shown that IGFBP-3 induces apoptosis in MCF-7 breast cancer cells by inhibiting IGF-I/Akt survival pathway [39]. Previous studies also indicated that IGFBP-3 can bind to a new cell death receptor (IGFBP-3R), a single-span membrane protein which specifically binds to IGFBP-3 but doesn't bind to other IGFBP's. *Invivo* studies using prostate and breast cancer xenografts in athymic nude mice, showed anti-tumor effects of IGFBP-3R [40]. It was shown from the *invitro* studies that IGFBP-3R triggers IGFBP-3 induced apoptosis in various cancer cells via a caspase-8 dependent pathway. IGFBP-3R directly interacts and activates caspase-8 in inducing apoptosis and knockdown of caspase-8 expression or activity can lead to inhibition of IGFBP-3/IGFBP-3R induced apoptosis. All these studies clearly indicate that IGFBP-3 induces its anti-proliferative and pro-apoptotic effects either by sequestering IGF-1 to prevent it to bind to Insulin-like growth factor-I receptor (IGF-IR) in an IGF dependent manner or by activating several candidate molecules via signal transduction pathway independent of IGF binding. Although Insulin-like growth factor binding protein-3 receptor (IGFBP-3R) has been known to be directly involved in IGFBP-3 action and may prove to be an important target molecule for the treatment of cancer, further studies are still needed to clarify the role of this receptor or other IGFBP-3 cell surface binding protein(s) in IGFBP-3 mediated cell signaling events.

4. Conclusions

IGFBP-3 is known to activate various downstream signaling molecules. Some of these signaling events could be initiated after binding to its receptor independent of IGF-I binding, to induce anti-proliferative and pro-apoptotic effects in certain type of cancer cells. On the other hand, IGFBP-3 can also bind to IGF-1 to modulate its ability to bind to IGF-1 receptor to inhibit cell proliferation and cell survival, resulting in cell death.

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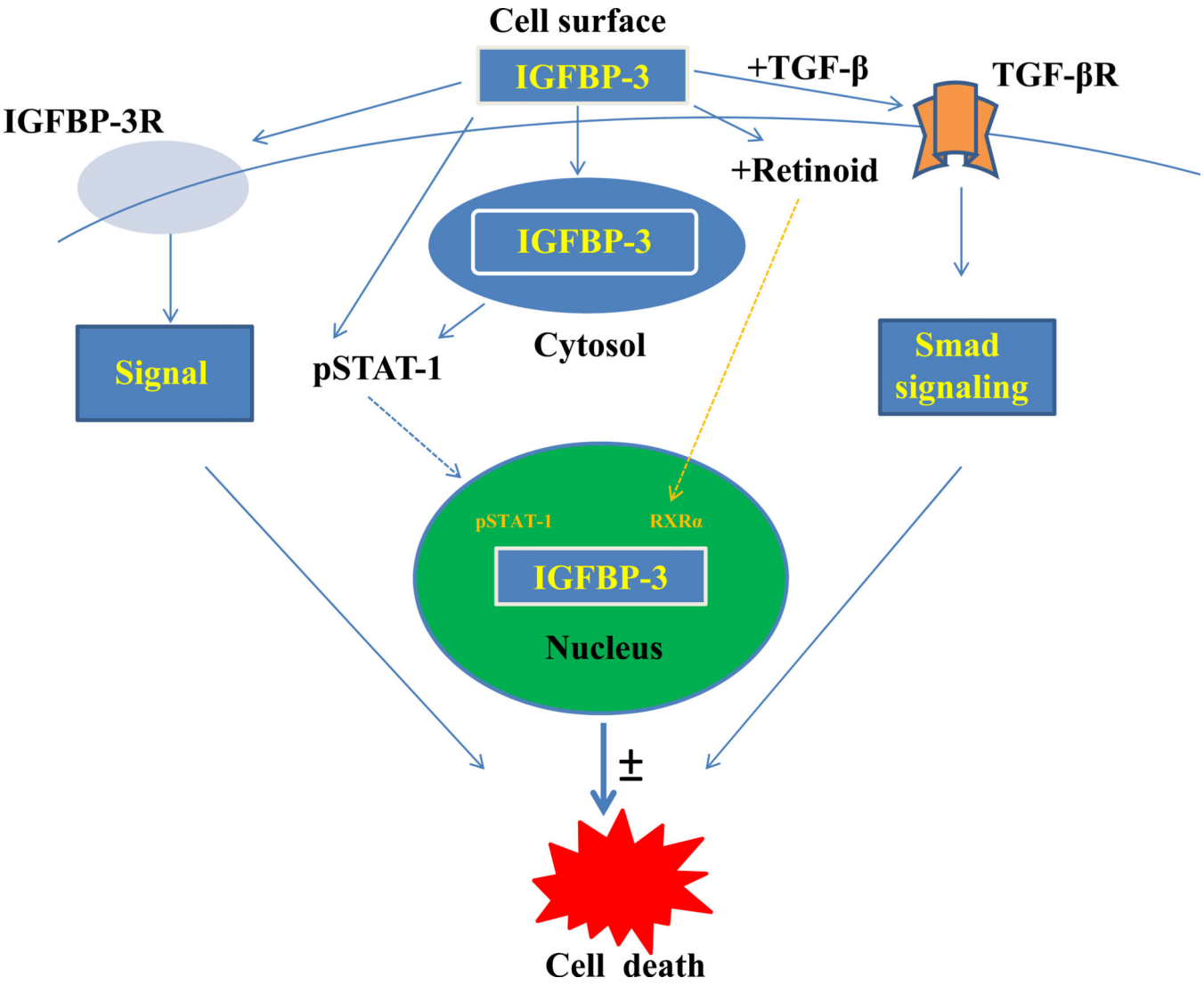

Figure 1. Key candidate molecules activated by IGFBP-3

IGFBP-3 can activate various downstream signaling molecules either by binding to its putative IGFBP-3R (receptor) to monitor its signal and induce apoptosis or bind to TGF-β receptor resulting in smad activation which leads to apoptosis. IGFBP-3 can also activate Stat-1 or bind to RXR-α to induce its anti-proliferative and pro-apoptotic effects.

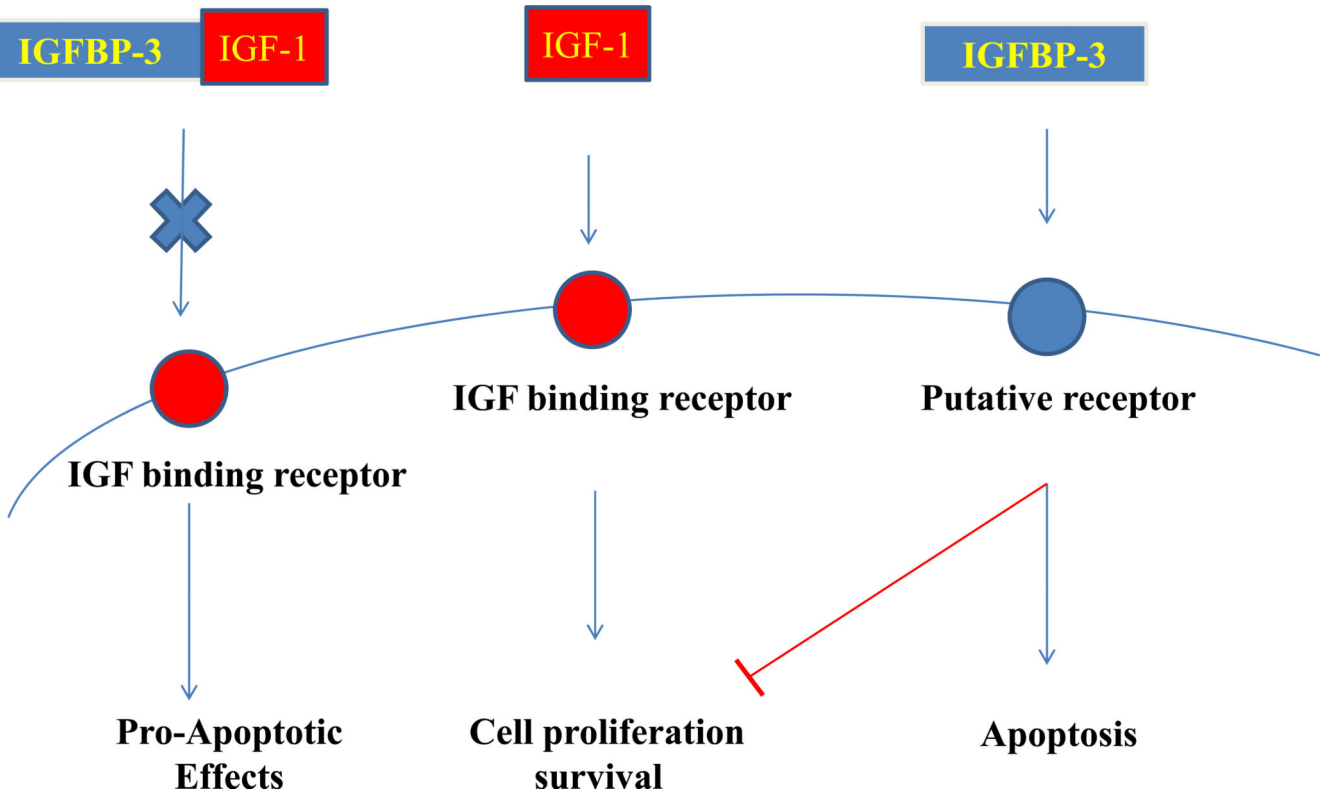

Figure 2. IGF dependent and Independent effects of IGFBP-3

IGF-1 can bind to IGF-I receptor and stimulate cell proliferation. IGFBP-3 blocks IGF-I to bind to its receptor resulting in the induction of apoptosis. IGFBP-3 can also induce apoptosis on its own by IGF-I independent mechanism.

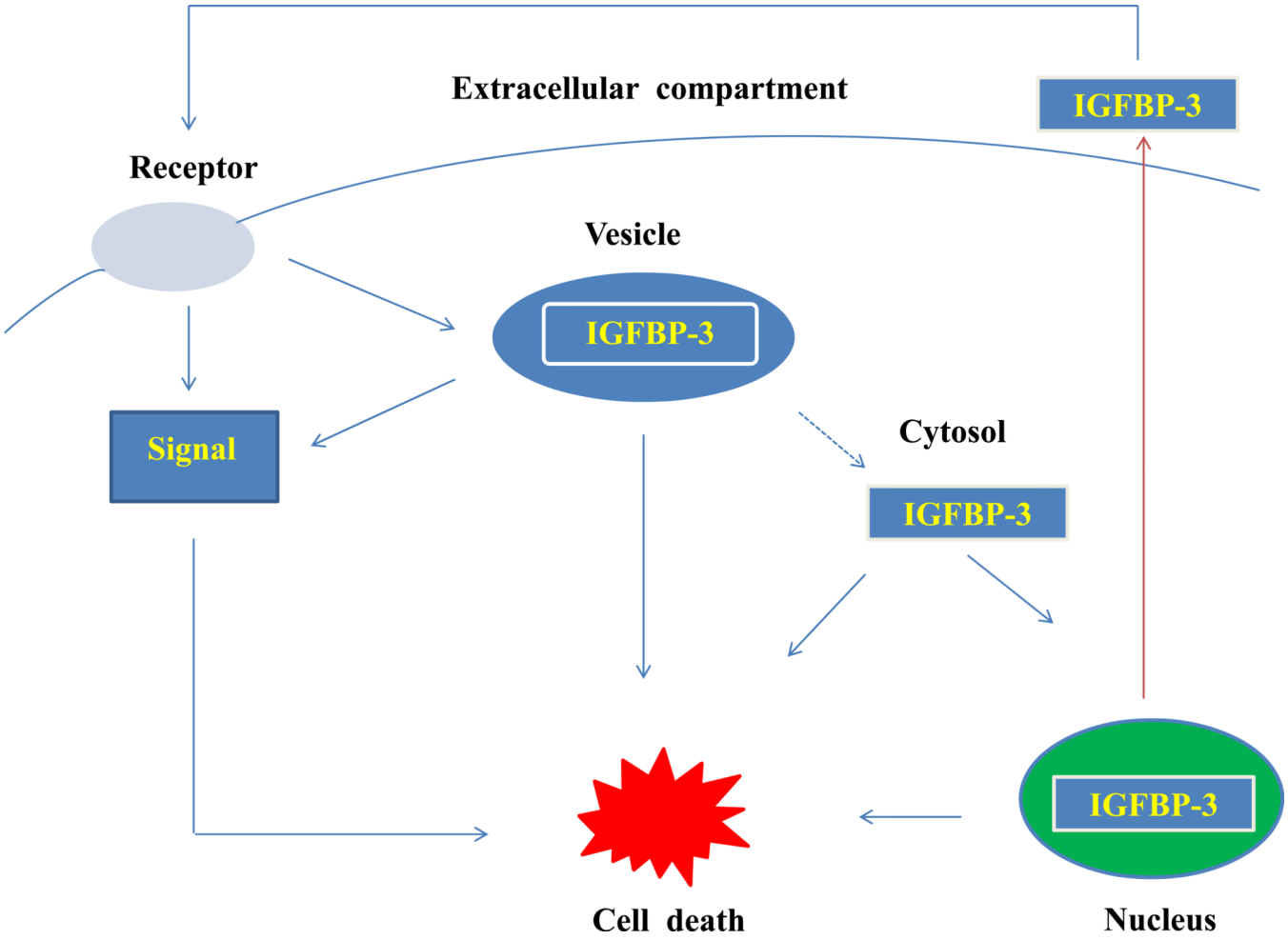

Figure 3. IGFBP-3 entry into the cell by different pathways

IGFBP-3 in the media seemingly binds with plasma membrane IGFBP-3 receptor to activate signal transduction pathway or enters into the cell by endocytosis to induce apoptosis. IGFBP-3 may either directly activate signal transduction pathway by binding to some receptor to induce apoptosis or in the form of vesicular IGFBP-3 which enters through Endoplasmic reticulum membrane into the cytosol and possibly gets translocated into the nucleus