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Mini review

Crosstalk between insulin-like growth factor (IGF) receptor and integrins through direct integrin binding to IGF1

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ABSTRACT

It has been generally accepted that integrin cell adhesion receptors are involved in growth factor signaling (integrin-growth factor crosstalk), since antagonists to integrins often suppress growth factor signaling. Partly because integrins have been originally identified as cell adhesion receptors to extracellular matrix (ECM) proteins, current models of the crosstalk between IGF1 and integrins propose that ECM ligands (e.g., vitronectin) bind to integrins and IGF1 binds to IGF receptor type 1 (IGF1R), and two separate signals merge inside the cells. Our research proves otherwise. We discovered that IGF1 interacts directly with integrins, and induces integrin-IGF-IGF1R complex formation on the cell surface. IGF1 signaling can be detected in the absence of ECM (anchorage-independent conditions). Integrin antagonists block both ECM-integrin interaction and IGF-integrin interaction, and do not distinguish the two. This is one possible reason why integrin-IGF1 interaction has not been detected. With these new discoveries, we believe that the direct IGF-integrin interaction should be incorporated into models of IGF1 signaling. The integrin-binding defective mutant of IGF1 is defective in inducing IGF signaling, although the mutant still binds to IGF1R. Notably, the IGF1 mutant is dominant-negative and suppresses cell proliferation induced by wt IGF1, and suppresses tumorigenesis in vivo, and thus the IGF1 mutant has potential as a therapeutic.

Contents

Abbreviations: CHO, Chinese hamster ovary; CSC, cancer stem cells; ECM, extracellular matrix; IGF1R, insulin-like growth factor type I receptor; IGF1, insulin-like growth factor-1; polyHEMA, poly-2-hydroxyethyl methacrylate.

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1. IGF-1/IGF1R axis is a therapeutic target of cancer

Insulin-like growth factor-1 (IGF1) is a polypeptide hormone that is homologous to proinsulin. Most of (80%) of the IGF1 in serum is synthesized by the liver and secreted, and it functions as an endocrine hormone. The remaining 20% of the IGF1 is synthesized in the periphery. Usually, connective tissue cell types, such as stromal cells, and IGF1 that is synthesized in the periphery can function to regulate cell survival by autocrine and paracrine mechanisms [1]. IGF1 is also secreted by many cancer cells at abnormally high levels. Once released by cancer cells, IGF1 binds and activates the type 1 IGF receptor (IGF1R) on their surface. IGF1R is ubiquitously present on multiple cell types. Ligand binding induces phosphorylation of specific tyrosine residues of IGF1R. These phosphotyrosines then bind to adapter molecules such as Shc and insulin receptor substrate-1. Phosphorylation of these proteins leads to activation of the phosphoinositide 3-kinase and mitogen activated protein kinase signaling pathways [2]. IGF1, thereby, confers cancer cells resistance to chemotherapy and radiation therapy. Thus, IGF1 is a major therapeutic target for cancer. Several strategies to target IGF1 signaling have been developed, including, siRNA and monoclonal antibodies for IGF-IR, and kinase inhibitors to inhibit the enzymatic activity of the receptor. The elucidation of the IGF1 signaling pathway (e.g., role of integrins, see below) should have a major impact in designing new therapeutic strategies [1].

1.1. IGF1R is a marker of cancer stem cells.

Several reports suggest that IGF1R is a marker of cancer stem cells (CSCs). IGF1R signaling is critical for maintaining "stemness" of CSCs, which is detected by the expression of Oct-4 and Nanog transcription factors. IGF1R is activated by IGF2, which is secreted by cancer-associated fibroblasts in a paracrine manner. IGF2 enhances expression of Oct-4 and Nanog in lung CSCs through IGF1R signaling and Akt phosphorylation (paracrine model)[3]. Development of new strategies to suppress IGF signaling should have a major impact in cancer treatment. IGF1 decoy is expected to inhibit IGF1R signaling in CSCs, and suppress stemness or the number of CSCs.

1.2. Previous studies on IGF1 signaling and integrin αvβ3

Integrins are a family of cell adhesion receptors that recognize extracellular matrix ligands and cell surface ligands [4]. They are transmembrane α-β heterodimers, and at least 18 α and 8 β subunits are known [5]. They are involved in signal transduction upon ligand binding [4]. Ligation of integrins also triggers a large variety of signal transduction events that serve to modulate cell behavior, including proliferation, survival/apoptosis, shape, polarity, motility, gene expression, and differentiation.

It has been well established that integrin αvβ3 plays a critical role in IGF signaling in addition to IGF1R [6–8]. "Ligand occupancy" of αvβ3 (*i.e.* the binding of extracellular matrix (ECM) proteins such as vitronectin to αvβ3) enhances signaling induced by IGF1 binding to IGF1R [1]. It has been proposed that ECM ligands bind to integrins and IGF1 binds to IGF1R, and two separate signals merge inside the cells. Blocking of vitronectin-αvβ3 integrin interaction using echistatin, a snake venom disintegrin that specifically inhibits αvβ3, inhibits IGF1-induced IGF1R phosphorylation, DNA synthesis [9] and cell migration [7]. The disulfide-linked loop of integrin β3 (The 177–184) in the ligand-binding site of this integrin is involved in vitronectin binding, and is necessary for IGF1 stimulated cell migration and proliferation [10]. The antibody against the disulfide-linked loop of β3 inhibits IGF1 signaling (IGF1-stimulated Shc phosphorylation and ERK1/2 activation) [10]. Anti-αvβ3 mAb and echistatin block IGF1-induced cell migration [11]. Also, echistatin blocked IGF1-stimulated DNA synthesis and IRS-1 phosphorylation, and attenuated IGF1R-linked down stream signaling events, such as activation of PI–3K and MAP kinase ERK1/2 [9]. We recently discovered that IGF1 interacts directly with integrins. Integrin antagonists block both ECM-integrin interaction and IGF-integrin interaction, and do not distinguish the two. This is one possible reason why integrin-IGF1 interaction has not been detected. *With these new discoveries*, we believe that the IGF-integrin interaction should be incorporated into models of IGF1 signaling.

1.3. Direct binding of IGF1 to integrin αvβ3 is critical for IGF signaling

The first indication that IGF1 binds to integrins is docking simulation, which we have been using to identify potential new integrin ligands. Docking simulation predicted that IGF1 binds to integrin αvβ3 well. We confirmed that this is really the case using human IGF1 we generated in our laboratory, and commercial IGF1 [12]. In surface plasmon resonance (SPR) analysis of IGF1- αvβ3 interaction using immobilized recombinant soluble αvβ3 to a sensor chip, we obtained KD 5×10^{-7} M, which is a reasonable affinity for integrin-ligand interaction. The simulation predicted the potential integrin-binding sites in IGF1 [12]. Mutating the Arg residues at positions 36/37 in the predicted integrin-binding site of IGF1 into Glu (designated R36E/R37E mutation) markedly reduced integrin binding of IGF1 [12]. The R36E/R37E mutant is defective in inducing IGF signaling (e.g., IGF1R phosphorylation, ERK1/2 activation, and cell proliferation), although the mutant still binds to IGF1R [12]. This suggests that the direct binding of integrins to IGF1 is critical for IGF signaling in addition to binding to IGF1R.

1.4. IGF1 induces integrin-IGF1-IGF1R ternary complex

Furthermore, WT IGF1 induces integrin-IGF1-IGF1R ternary complex formation, while R36E/R37E does not (Fig. 1a), suggesting that the ternary complex formation plays a role in IGF1 signaling [12]. Our studies show phosphorylation of IGF1R is not required for IGF1-induced integrin-IGF1R association. Inhibitors of IGF1R (PPP) and Src (PP2) does not suppress αvβ3-IGF-IGF1R ternary complex formation [13], suggesting that phosphorylation of IGF1R is not required for ternary complex formation. We propose a model, in which IGF1 binding to IGF1R induces recruitment of integrin αvβ3 to the IGF-IGF1R complex, and then β3 and IGF1R are phosphorylated [13] (Fig. 1b). It is likely that αvβ3 is together with the IGF1-IGF1R complex for triggering IGF signaling.

1.5. IGF1-induced physical association of integrin β3 and IGF1R is detected by BiFC and FRET studies

The association of αvβ3 and IGF1R has recently been studied in bimolecular fluorescence complementation (BiFC) studies using β3 tagged with YFP1-158 and IGF1R tagged with YFP159-299 [14]. αvβ3 and IGF1R did not interact on the cell surface, but WT IGF1 potentiated the association. Essentially the same results were obtained in FRET studies (CFP-tagged integrin β3 and YFP tagged