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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.

Keywords

IGF1; integrin; crosstalk; dominant-negative antagonist; signaling

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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].

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.

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.

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.

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.

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 IGF1R) [14]. These findings are consistent with our biochemical findings that IGF1 binding to αvβ3 induces ternary complex formation with IGF1R.

IGF1 signaling, integrin α6β4, and cancer

The α6β4 integrin is a receptor for the laminin family of extracellular matrix proteins, and its expression is associated with poor patient prognosis and reduced survival in a variety of human cancers [15]. α6β4 combines with multiple oncogenic receptor tyrosine kinases, such as ErbB2 [16], EGFR [17], and Met [18] and enhances the signaling function. However, how α6β4 and RTKs interact is not well understood. α6β4 in normal epithelial cells is located in basal surface of the cells, and α6β4 is relocated to the apical region of the cells when cells were transformed or stimulated with EGF [19]. The change in α6β4 localization is accompanied by the loss of polarity and shape of the epithelial cells. It is expected that α6β4 is exposed to growth factors when it moves from the basal surface of the cells, in which α6β4 is shielded from growth factors, to the apical region.

The β4 subunit was originally identified as a tumor-related antigen expressed in metastatic cancer [20]. In contrast with its function in regulating stable adhesion through the formation of hemidesmosomes in normal epithelial cells, α6β4 promotes motility and invasion in carcinoma cells [21]. Moreover, suppression of α6β4 expression by siRNA diminishes invasive potential [22]. α6β4 expression has been correlated with the progression and metastatic potential of several different tumors including breast cancer and pancreatic cancer [23,24,25]. α6β4 is up regulated in various types of cancer (squamous, breast, thyroid, bladder, colorectal, and gastric)[19]. In addition, α6β4 is expressed in tumor blood vessels and promotes the invasion phase of tumor angiogenesis [26].

We demonstrated that integrin α6β4 directly binds to IGF1. WT IGF1 induced ternary complex formation (α6β4-IGF1-IGF1R) in MCF-7 breast cancer cells, but R36E/R37E IGF1 did not (see below), suggesting that α6β4-IGF1 interaction plays a role in IGF1 signaling possibly through integrin-IGF1R crosstalk.

Overexpression of α6β4 enhances, and down regulation of α6β4 suppresses, proliferation of MCF-7 cells [27,15], suggesting that α6β4 plays a role in proliferation of this cell type. We found that α6β4 binds to IGF1 at a level comparable to that of αvβ3. Interestingly, α6β4 does not adhere to R36E/R37E, suggesting that α6β4 binds to IGF1 in a manner similar to that of αvβ3. Also, a6β4 co-precipitated with wt IGF1 but not well with R36E/R37E, suggesting that α6β4 specifically recognize IGF1. We found that WT IGF1 induced co-precipitation of IGF1R and integrin β4, but R36E/R37E was defective in this function. This

suggests α6β4-IGF1-IGF1R ternary complex formation is involved in IGF signaling in cancer cells.

The R36E/R37E IGF1 mutant suppresses cell proliferation, anchorage-independent growth (in soft agar), and tumorigenesis

We stably expressed WT and mutant IGF1 in mouse and human breast cancer cells using secretion vector. WT IGF1 enhances proliferation of stably transfected cells, while R36E/R37E suppresses it. We tested if WT and mutant IGF1 affects anchorage-independent growth, an important hallmark of cell transformation, of cancer cells. Interestingly, WT IGF1 enhances the number of colonies in soft agar, while R36E/R37E slightly reduced it. We studied if IGF1 can induce signaling in anchorage-independent conditions in transformed cells (Chinese hamster ovary, CHO) that express αvβ3 (β3-CHO) cells. We found that IGF1 signals were more clearly detectable in anchorage-independent conditions (polyHEMA-coated plates) than in regular tissue culture conditions. This suggests that IGF signaling is masked by signals from cell-matrix interaction in regular tissue culture. IGF signaling required αvβ3 expression, and R36E/R37E was defective in inducing signals in polyHEMA-coated plates. These results suggest that αvβ3-IGF1 interaction, not αvβ3-extracellular matrix interaction, is essential for IGF signaling.

We tested whether R36E/R37E IGF1 affects tumorigenesis in vivo using cancer cells that constitutively secrete WT IGF1 or R36E/R37E. R36E/R37E suppresses tumorigenesis in vivo, while WT IGF1 enhances it. This is consistent with the results in vitro that R36E/R37E is dominant-negative.

We found that excess R36E/R37E suppresses the binding of labeled-WT IGF1 to the cell surface [28], and suppresses ternary complex formation induced by WT IGF1 [28]. This demonstrates that R36E/R37E competes with WT IGF1 for the binding to IGF1R on the cell surface.

IGF signaling occurs in anchorage-independent conditions

It has been proposed that integrin-ECM interaction is required for signaling from growth factors [29,30]. In IGF signaling, “ligand occupancy” of αvβ3 through vitronectin binding to αvβ3 enhances signaling induced by IGF1 binding to IGF1R [1]. We found, however, that exogenous WT IGF1 did not induce detectable signaling in regular tissue culture (2-dimensional culture) [13]. We hypothesized that massive proliferative signals from cell-ECM interaction masks the signals from exogenous IGF1. We thus suppressed cell-ECM interaction by coating the plastic surface with hydrogel (polyHEMA), which generate anchorage-independent conditions [31]. We also used soft agar plates, a well-established anchorage-independent condition. Under these conditions we detected the effect of exogenous WT IGF1, the dominant-negative effect of R36E/R37E, and the effect of αvβ3 or α6β4 expression [32]. (Fig. 2)

These findings are consistent with a previous report that cell-matrix adhesion masks the heparin-binding EGF signaling because cell-matrix adhesion provides cells sufficient

proliferative signals through cell-matrix adhesion, but that it is possible to detect the proliferative effect of heparin binding EGF on cancer cells in vitro in three- or two-dimensional culture in which cell-matrix interaction is reduced [33]. Thus it is unclear if cell-ECM interaction really enhances IGF1 signaling because it masks the effect of exogenous IGF1 signaling. It is likely that exogenous IGF may not be required for proliferation or survival when cells interact with ECM. If tumor growth in anchorage-independent conditions, in which cell-ECM interaction is reduced, mimics tumorigenesis in vivo, it is highly likely that IGF1-integrin interaction plays a major role in tumorigenesis rather than integrin-ECM interaction. Thus we will need to study how ECM and IGF (and perhaps other cytokines) contribute to proliferation and cell survival in future studies.

Can mutants of human proteins be used as therapeutic agents?

There is a precedent that a mutant of human protein was used for human diseases. A mutant of human growth hormone (hGH) has been used as an antagonist of GH receptor in the treatment of acromegaly (Pegmicovent) [34]. The Gly-120 of h GH was mutated to Arg (G120R) and this mutant was further modified by polyethyleneglycol (PEG)-5000 to elongate half-life. Pegvisomant prevents functional dimerization of hGH receptor by sterically inhibiting conformational changes within the GHR dimers [34]. Pegvisomant is generally well tolerated with a safety profile similar to that reported in clinical trials and can effectively reduce IGFI in patients with acromegaly refractory to conventional therapy [35].

Integrin-growth factor crosstalk through direct integrin binding to growth factor is not limited to IGF1

Our previous studies found that FGF1directly bind to integrins using docking simulation [36]. Based on the docking model, we generate integrin-binding-defective FGF1 by introducing mutations in the predicted integrin-binding site. The integrin-binding-defective mutant (the R50E mutant) is not only defective in inducing signaling, it also suppresses FGF signaling induced by wild-type FGF1 (dominant-negative effect by definition) [37]. Wild-type FGF1 induces ternary complex formation (integrin-FGF-FGF receptor), but R50E does not. WT FGF1 induces ternary complex formation (integrin, FGF1, and FGF receptor-1 (FGFR1), when cells are treated with FGF1. The integrin-binding-defective mutant of FGF1 does not induce ternary complex formation, suggesting that the ability of FGF1 to interact with integrins is required for FGF signaling. Notably, we found that the integrin-binding defective mutant of FGF1 acts as an antagonist to FGF signaling (dominant-negative mutants) [37,38], suggesting that the FGF mutant has potential as a therapeutic. We described very similar findings in neuregulin-1 [39], and fractalkine [40]. Thus integrin-growth factor crosstalk through direct integrin binding to growth factor is not limited to IGF1.

Conclusion

Our studies suggest that integrin binding to IGF1 and subsequent ternary complex formation is required for IGF1 signaling (Fig. 1). The integrin-binding defective IGF1 mutant is an interesting tool for studying the mechanism of integrin-IGF1 crosstalk. The dominant-

negative IGF1 mutant has potential as a therapeutic in cancer. Thus we need to fully evaluate the potential of the IGF1 mutant as a therapeutic agent. How can we deliver the dominant-negative IGF1 mutant to diseased tissues? Small devices that deliver insulin to diabetic patients have been developed, in which timing and amount of delivery are fully programmable. One possibility is that we will be able to exploit these devices for delivering IGF1 instead of insulin.

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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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Biographies

Yoshikazu Takada:

I obtained MD and PhD degrees in Japan, and studied liver peroxisomes in animals for 10 years, I moved to Dana-Farber cancer institute in Boston in 1986 and was involved in identification of b1 integrins. I moved to the Scripps Research Institute in La Jolla in 1990,

and then UC Davis School of Medicine (2003-present) and at the PhD program for translational medicine, Taipei Medical University. Current interest is integrin-growth factor crosstalk and integrin signaling. Using docking simulation, we identified several new integrin ligands including FGF, IGF, neuregulin, fractalkine, secreted phospholipase A2 type IIA, and C-reactive protein. Our studies aim to identify new integrin functions that are not related cell adhesion to ECM.

Masaaki Fujita:

I studied chemistry, structural biology, and protein engineering at Tokyo Institute of Technology in Tokyo, Japan. I then completed a MD/PhD program at Kobe University School of Medicine, Kobe Japan. I conducted clinical and basic research in the field of rheumatology and immunology during residency. I spent several years as a postdoctoral scholar and then assistant researcher at UC Davis, School of Medicine, Sacramento, USA. I was involved in the project on integrin-growth factor crosstalk during this period. Currently, I am a physician scientist (vice chair of Department of Clinical Immunology and Rheumatology) at The Tazuke-Kofukai Medical Research Institute, Kitano Hospital in Osaka, Japan. I intend to connect basic science and medicine and advance bench to bedside research.

Highlights

- IGF1 directly binds to integrins and induces ternary complex formation (integrin, IGF1, and IGF1R) on the cell surface. The integrin-binding defective IGF1 mutant is defective in inducing IGF signaling, although the mutant still binds to IGF1R.
- The integrin-binding defective IGF1 mutant is dominant-negative and suppresses cell proliferation induced by wt IGF1, and suppresses tumorigenesis in vivo.
- IGF1 signaling is detected in anchorage-independent conditions (in the absence of integrin-ECM interaction), suggesting that integrin-IGF1 interaction, rather than integrin-ECM interaction, is important for IGF signaling.
- In addition to IGF1, several other cytokines (FGF, neuregulin-1, fractalkine) directly bind to integrins and induce ternary complex formation, suggesting that integrins act as co-receptors common to these cytokines.

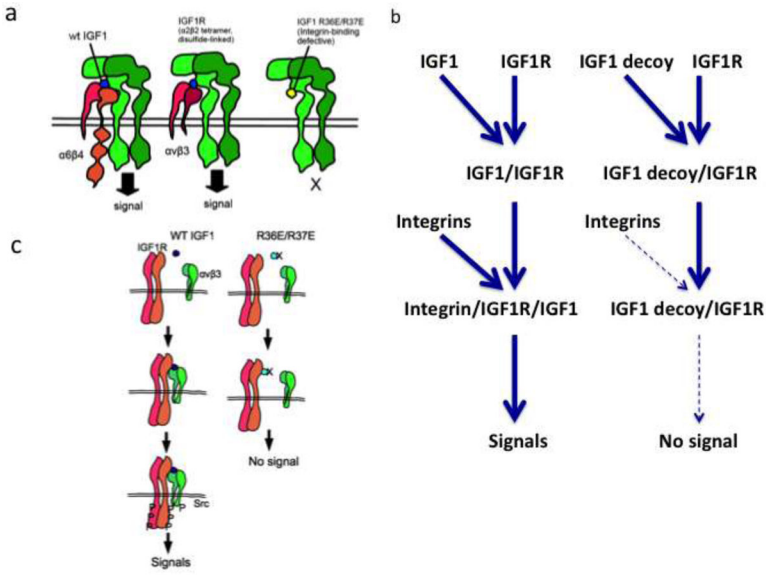

Fig. 1.

A model of IGF signaling. IGF1 binds to IGF1R, and then integrin αvβ3 (and α6β4 and perhaps other integrins as well) is recruited to the IGF1- IGF1R complex. a. Our studies found that WT IGF1 directly binds to integrins and induces ternary complex formation on the cell surface. The integrin-binding defective mutant (R36E/R37E) of IGF1 is defective in inducing signals and ternary complex formation while the mutant still binds to IGF1R. b. IGF1 binds first its cognate high-affinity receptor IGF1R, and then integrins are recruited to the IGF1/IGF1R complex, resulting the formation of integrin-IGF1-IGF1R ternary complex. The integrin-binding defective IGF1 mutant (R36E/R37E, IGF1 decoy) generates an inactive IGF1R/IGF1 decoy complex upon binding to IGF1R, and integrins cannot be recruited to the IGF1R/IGF1 decoy complex. c. Does ternary complex formation require IGF1R activation? Our studies found that PPP, a specific IGF1R inhibitor, did not block the ternary complex formation, indicating that integrin-IGF1-IGF1R ternary complex formation does not require IGF1R phosphorylation. We speculate that ternary complex formation happens before IGF1R is phosphorylated. P= phosphate.

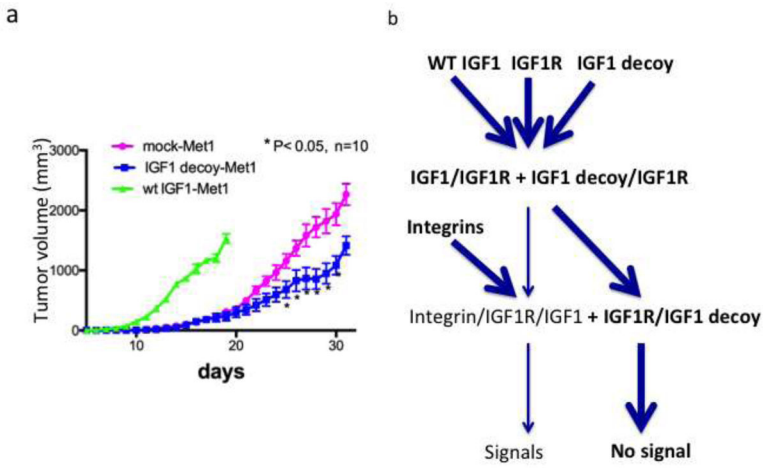

Fig. 2.

R36E/R37E suppresses cell viability and tumorigenesis, whereas WT IGF1 enhances cell viability and tumorigenesis. Met-1 cells that secrete WT or IGF1 decoy (R36E/R37E) were injected into the mammary fat pads of FVB mice. WT IGF1 markedly enhanced tumor growth, but IGF1 decoy delayed tumor growth. b. A potential mechanism of the antagonistic action of IGF1 decoy. IGF1 decoy competes with WT IGF1 for binding to IGF1R and suppresses the ternary complex formation on the cell surface, resulting in the inactive IGF1R/IGF1 decoy complex.