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FEBS 28327 FEBS Letters 565 (2004) 117–121

Cellular growth inhibition by TGF-β_1 involves IRS proteins ☆

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Received 1 March 2004; revised 24 March 2004; accepted 24 March 2004

First published online 9 April 2004

Edited by Veli-Pekka Lehto

Abstract In Mv1Lu cells, insulin partially reverses transforming growth factor-β_1 (TGF-β_1) growth inhibition in the presence of α5β1 integrin antagonists. TGF-β_1 appears to induce phosphorylation of IRS-2 in these cells; this is inhibited by a TGF-β antagonist known to reverse TGF-β growth inhibition. Stable transfection of 32D myeloid cells (which lack endogenous IRS proteins and are insensitive to growth inhibition by TGF-β_1) with IRS-1 or IRS-2 cDNA confers sensitivity to growth inhibition by TGF-β_1; this IRS-mediated growth inhibition can be partially reversed by insulin in 32D cells stably expressing IRS-2 and the insulin receptor (IR). These results suggest that growth inhibition by TGF-β_1 involves IRS proteins.
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Keywords: Transforming growth factor-β; Growth inhibition; Type V transforming growth factor-β receptor; Insulin receptor substrate protein; Insulin; α5β1 integrin

1. Introduction

Transforming growth factor-β (TGF-β) is a family of structurally homologous dimeric proteins (three mammalian isoforms: TGF-β_1, TGF-β_2 and TGF-β_3) [1,2]. TGF-β is a bifunctional growth regulator; it inhibits growth of most cell types, including epithelial, endothelial and hematopoietic cells, but stimulates growth of mesenchymal cells such as fibroblasts and osteoblasts [3]. In addition to its growth regulatory activities, TGF-β exhibits other biological activities, including regulation of extracellular matrix synthesis, chemotaxis, angiogenesis and differentiation of several cell lineages. It has been implicated in many pathophysiological processes including wound repair, tissue fibrosis, immunosuppression and morphogenesis [4].

Two of its prominent biological activities are cell growth inhibition and transcriptional activation of extracellular matrix synthesis-related genes. Accumulating evidence indicates that these two activities are uncoupled in many human carcinoma cells [5–10]. Such cells fail to respond to growth inhibition by TGF-β but exhibit TGF-β-mediated transcriptional activation of extracellular matrix synthesis-related genes; this is known to be primarily mediated by the type I/type II TGF-β receptor (TβR-I/TβR-II) heterocomplex in the cell systems studied so far [11,12]. The separation of these activities implies that signaling pathways other than the TβR-I/TβR-II signaling cascade are involved [10,13–16]. Several signaling cascades, which are different from the TβR-I/TβR-II heterocomplex/Smad2/3/4 signaling cascade, have been shown to be involved in the growth inhibitory response to TGF-β [10,13–16]. However, it is not known which TGF-β receptor types mediate the activation of these signaling cascades because most of the cell systems used for the investigations express other TGF-β receptor types in addition to TβR-I and TβR-II.

The type V TGF-β-receptor (TβR-V) is a high molecular weight non-proteoglycan membrane protein and co-expresses with TβR-I, TβR-II and TβR-III in most cell types [17–21]. Many human carcinoma cells express little or no TβR-V [19–22], and their growth is not inhibited by TGF-β. This suggests that TβR-V may be involved in the growth inhibitory response to TGF-β and that its loss may contribute to the malignant phenotype of these human carcinoma cells. The identification of TβR-V as the IGFBP-3 receptor, which mediates the IGF-independent (TGF-β peptide antagonist sensitive) growth inhibitory response to IGFBP-3, has highlighted the likely importance of TβR-V in mediating the growth inhibitory response [6,21,22]. TβR-V was also recently found to be identical to low density lipoprotein receptor-related protein-1 (LRP-1) [23]. Several lines of evidence have revealed that TβR-V/LRP-1 is required for growth inhibition by IGFBP-3 and TGF-β [23]. The finding that TβR-V is identical to LRP-1 has disclosed previously unreported growth regulatory function of LRP-1. LRP-1 is best known as an endocytic receptor [24,25]. Increasing evidence indicates that LRP-1 is also capable of mediating signaling [24,25]. However, the molecular mechanisms by which LRP-1 mediates signaling are not understood [24,25]. Recently, we found that TβR-V/LRP-1-mediated growth inhibition by IGFBP-3 requires insulin receptor substrate proteins (IRSs) [26]. Since TβR-V/LRP-1 also mediates growth inhibition by TGF-β_1 [23], we investigated the function of IRSs in TGF-β_1-induced growth inhibition. In this communication, we demonstrate that IRSs are involved in growth inhibition by TGF-β_1.

2. Materials and methods

2.1. Materials

[^{32}P] Orthophosphate (500 mCi/ml) and [methyl-^{3}H] thymidine (67 Ci/mmol) were purchased from ICN Biochemicals (Irvine, CA). Anti-IRS-2 IgG was obtained from Santa Cruz Biotech (Santa Cruz, CA). Anti-α5β1 integrin serum (rabbit) was obtained from Chemicon

☆ This work was supported by National Institutes of Health Grant CA 38808.

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doi:10.1016/j.febslet.2004.03.082

International, Inc. (Temecula, CA). Protein molecular mass standards (250, 148, 98, 64, 50, and 36 kDa) were obtained from Invitrogen (Carlsbad, CA). Protein A–Sepharose was obtained from Pharmacia LKB Biotech (Piscataway, NJ). $\beta_1{}^{25}(41\text{–}65)$, a TGF-β peptide antagonist, was prepared as described previously [27]. Human TGF-β_1 was purchased from Austral Biologicals (Santa Clara, CA) and R&D Systems (Minneapolis, MN). Cyclo (GRGDSPA) was obtained from Bachem Bioscience (King of Prussia, PA). Human IGFBP-3 (expressed in *E. coli*, M.W. ~35 000) and anti-IRS-2 IgG were obtained from Upstate (Charlottesville, VA). Murine 32D myeloid cells expressing vector only and 32D/IRS-1 and 32D/IR/IRS-2 cells, which were stably transfected with human IRS-1 cDNA and the insulin receptor (IR)/IRS-2 cDNAs, respectively, were provided by Dr. Martin G. Myers, Jr., Joslin Diabetes Center, Harvard University, Boston, MA. Wild-type 32D cells did not express IRS-1 and IRS-2 as demonstrated by RT-PCR and immunoblot analyses [28]. 32D/IRS-1 and 32D/IR/IRS-2 cells expressed comparable levels of IRS-1 and IRS-2, respectively, as demonstrated by immunoblot analysis [29].

2.2. ^{32}P-metabolic labeling and immunoprecipitation

^{32}P-metabolic labeling and immunoprecipitation of IRS-2 was performed as described previously [26]. Mv1Lu cells were grown in Dulbecco's modified Eagle's medium (DMEM), changed to phosphophate-free DMEM containing 0.2% of dialyzed fetal calf serum for 1 h and labeled with ^{32}P-orthophosphate (200 μC/ml) for 2 h. They were then treated with increasing concentrations of TGF-β_1 in the presence and absence of IGFBP-3 (1 μg/ml) for 2.5 h. The cells were lysed in RIPA buffer (1% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 25 mM Tris–HCl, pH 7.4, and 0.15 M NaCl). Equal amounts of protein (200 μg) from the cell lysates of ^{32}P-labeled cells were immunoprecipitated with anti-IRS-2 IgG. The immunoprecipitates were analyzed by 7.5% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions and autoradiography (quantitated with a PhosphoImager). The ^{32}P-labeled IRS-2 was excised from the dried gel and subjected to phosphoamino acid analysis as described previously [20].

2.3. [Methyl-^{3}H] thymidine incorporation assay

[Methyl-^{3}H] thymidine incorporation assay was performed according to our published procedures [18–21]. Mv1Lu cells were plated on 48-well clustered dishes (cell density: $1\text{–}2 \times 10^5$/well) and incubated with TGF-β_1 (0.25 pM) in the presence of increasing concentrations of insulin in DMEM containing 0.2% fetal calf serum. After incubation at 37 °C for 18 h, [methyl-^{3}H] thymidine incorporation into cellular DNA was determined as described previously [6]. 32D cells were grown in RPMI 1640 medium containing 10% fetal calf serum and 5% WEHI conditioned medium (which contained IL-3) [26]. These cells were treated with increasing concentrations of TGF-β_1 with or without insulin (10 nM) in RPMI 1640 medium containing 0.2% fetal calf serum and 0.05% WEHI conditioned media. After incubation at 37 °C for 8 h, [methyl-^{3}H] thymidine incorporation into cellular DNA was determined [26]. The assays were performed in quadruplicate.

3. Results

3.1. Insulin partially reverses growth inhibition by TGF-β_1 in the presence of α5β1 integrin antagonists

Since insulin had been shown to reverse TβR-V/LRP-1-mediated growth inhibition by IGFBP-3 [26], we examined the effect of insulin on TGF-β_1-induced growth inhibition in Mv1Lu cells. These cells were treated with 0.25 pM TGF-β_1 in the presence of increasing concentrations of insulin. After 18 h at 37 °C, DNA synthesis was determined by measuring [methyl-^{3}H] thymidine incorporation into cellular DNA. As shown in Fig. 1A, insulin up to 70 nM did not significantly affect DNA synthesis inhibition induced by 0.25 pM TGF-β_1. As a positive control, Mv1Lu cells were treated with 0.2 μg/ml of IGFBP-3 in the presence and absence of insulin (7 nM). IGFBP-3 (0.2 μg/ml) inhibited DNA synthesis by 36% in these cells. This IGFBP-3-induced DNA synthesis inhibition was completely reversed in Mv1Lu cells co-treated with insulin (7 nM). These results suggest that, although TβR-V mediates growth inhibition by both IGFBP-3 and TGF-β_1, the mechanism by which TGF-β_1 induces growth inhibition is more complex than that for IGFBP-3-induced growth inhi-

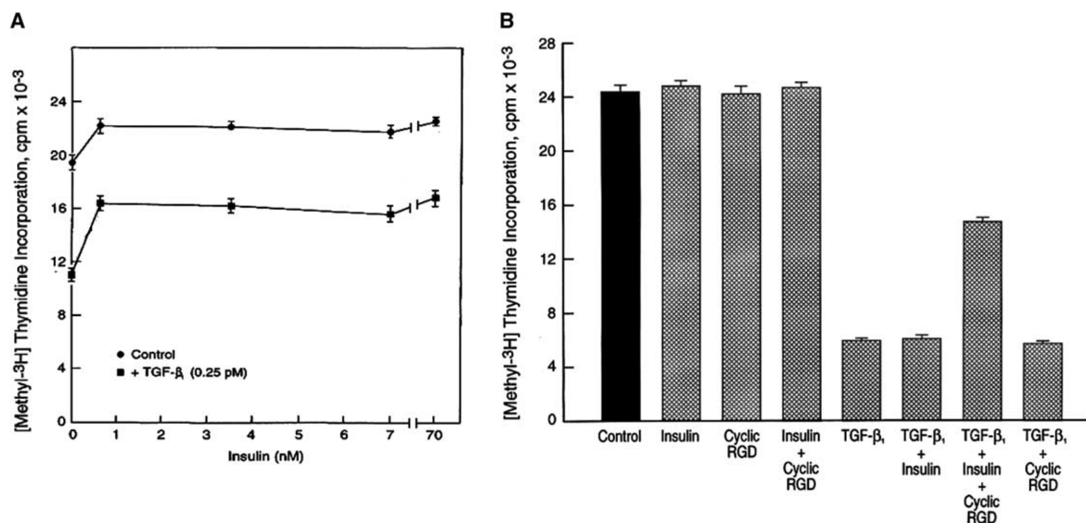

Fig. 1. Effects of insulin on DNA synthesis in Mv1Lu cells treated with TGF-β_1 in the absence (A) or presence (B) of a cyclic RGD peptide. (A) Cells were treated with 0.25 pM TGF-β_1 in the presence of increasing concentrations (as indicated) of insulin. After 18 h at 37 °C, DNA synthesis was determined by measuring [methyl-^{3}H] thymidine incorporation into cellular DNA. Each data point is the mean ± S.D. of quadruplicate determinations in four independent experiments. As a positive control, Mv1Lu cells were treated with 0.2 μg/ml of IGFBP-3 in the presence and absence of insulin (7 nM). IGFBP-3 (0.2 μg/ml) inhibited DNA synthesis by 36% in these cells, which was completely reversed by 7 nM insulin. (B) Cells were treated with and without TGF-β_1 (0.5 pM) ± insulin (10 nM) ± a cyclic RGD peptide (Cyclo GRGDSPA, 0.01 μg/ml) for 18 h at 37 °C. The [methyl-^{3}H] thymidine incorporation into cellular DNA was determined. The bars represent means ± S.D. of triplicate determinations in four independent experiments. The DNA synthesis inhibition (~39%) in cells treated with TGF-β_1, Cyclo GRGDSPA and insulin was significantly less than that (~75%) in cells treated with TGF-β_1 alone or TGF-β_1 + insulin or Cyclo GRGDSPA (Student's t test, $P < 0.001$).

bition. The IGFBP-3-induced growth inhibition is mainly mediated by TβR-V/LRP-1, whereas TβR-V/LRP-1 mediates TGF-β growth inhibition in concert with TβR-I and TβR-II [7,23,26].

Gagnon et al. [30] reported that both extracellular matrix expression induced by TGF-β_1 and fibronectin impair insulin-induced signal transduction by inhibiting insulin-dependent IRS tyrosine phosphorylation. Thus, the ability of insulin to block the TβR-V-mediated growth inhibitory response to TGF-β_1 may be impaired by the extracellular matrix induced by TGF-β_1 (which is mediated by the TβR-I/TβR-II heterocomplex). To test this possibility, we examined the effect of insulin on growth inhibition by TGF-β_1 (as determined by measurement of DNA synthesis) in the presence and absence of a cyclic RGD peptide (cyclo GRGDSPA) [29] which blocks the binding of extracellular matrix proteins (e.g., fibronectin) to their receptors, (e.g., α5β1 integrin) [31]. As shown in Fig. 1B, TGF-β_1 (0.5 pM) inhibited ~75% of DNA synthesis in Mv1Lu cells. Neither insulin (10 nM) alone nor the cyclic RGD peptide (0.01 μg/ml) alone affected TGF-β_1 inhibition of DNA synthesis. However, insulin was able to partially reverse the TGF-β_1 inhibition in the presence of the cyclic RGD peptide. The combination of insulin and the cyclic RGD peptide decreased the TGF-β_1-induced DNA synthesis inhibition from ~75% to ~39%. This suggests that insulin not only is capable of blocking IGFBP-3 growth inhibition [26], but also can block growth inhibition by TGF-β_1 under certain conditions, such as when a cyclic RGD peptide blocks binding of fibronectin to α5β1 integrin. This suggestion is further supported by the observation that insulin also partially reverses growth inhibition caused by TGF-β_1 in the presence of anti-α5β1 integrin serum. As shown in Table 1, the combination of insulin plus anti-α5β1 integrin serum decreased the DNA synthesis inhibition induced by TGF-β_1 from ~40% to ~15% in Mv1Lu cells. Neither insulin alone, anti-α5β1 integrin serum alone nor non-immune serum alone had any significant effect on DNA synthesis in cells treated with or without TGF-β_1.

3.2. TGF-β_1 induces serine-specific phosphorylation of IRS-2 in Mv1Lu cell

Mv1Lu cells are a standard model cell system for investigating growth inhibition by TGF-β and IGFBP-3 [6,21–23,32]. Since IRS proteins are required for growth inhibition by IGFBP-3 and since IGFBP-3 induces serine-specific dephosphorylation of IRS-2 [26], it would be important to observe the effect of TGF-β_1 on the phosphorylation status of IRS-2 in these cells. As shown in Fig. 2A, TGF-β_1 at 100 pM induced phosphorylation of IRS-2 by ~2-fold (lane 3 versus lane 1). This TGF-β_1-stimulated phosphorylation was inhibited by $\beta_1{}^{25}$(41–65), a TGF-β peptide antagonist (Fig. 2A, lane 2 versus lane 3). $\beta_1{}^{25}$(41–65) is known to reverse TGF-β growth inhibition by blocking TGF-β binding to TGF-β receptors [23,26,27]. $\beta_1{}^{25}$(41–65) alone did not influence phosphorylation of IRS-2 (data not shown, [26]). Phosphoamino acid analysis revealed that both TGF-β-unstimulated and -stimulated phosphorylation occurred at serine residues as previously reported [26]. Since Mv1Lu cells expressed TβR-I and TβR-II, both of which are Ser/Thr-specific protein kinases, the TGF-β_1-induced phosphorylation may be mediated by TβR-II or TβR-II/TβR-I complexes. To test this possibility, we examined the effect of increasing concentrations of TGF-β_1 on IRS-2 phosphorylation in DR26 cells, which are Mv1Lu mutant cells lacking functional TβR-II. As shown in Fig. 2B, increasing concentrations of TGF-β_1 correspondingly stimulated phosphorylation (^{32}P-labeling) of IRS-2 in these cells (DR26 cells) (Fig. 2B, lanes 3, 5, 8, 10 versus lane 1). Interestingly, the TGF-β_1-induced phosphorylation of IRS-2 was overridden by IGFBP-3-induced dephosphorylation in these cells (Fig. 2B, lanes 4, 6, 7, 9 versus lanes 3, 5, 10, 8, respectively). These

Table 1
Effect of insulin on DNA synthesis in Mv1Lu cells treated with TGF-β_1 in the presence of anti-α5β1 integrin serum

	[Methyl-^{3}H] thymidine incorporation*	
	cpm/well	%
Control	153 811 ± 4415	100
+TGF-β_1	89 367 ± 7307	58
+TGF-β_1 + insulin	94 535 ± 3426	61
+TGF-β_1 + anti-α5β1 integrin	92 454 ± 3943	60
+TGF-β_1 + insulin + anti-α5β1 integrin	130 493 ± 3616	85**
+insulin	154 844 ± 2436	100
+anti-α5β1 integrin	157 983 ± 4439	100

*Cells (~5 × 10^4 cells/well) were treated with or without TGF-β_1 (1 pM) ± insulin (10 nM) ± anti-α5β1 integrin serum or control non-immune serum (400× dilution) for 18 h. DNA synthesis was determined by measuring [methyl-^{3}H] thymidine incorporation into cellular DNA. The [methyl-^{3}H] thymidine incorporation in cells treated with TGF-β_1 + insulin + non-immune serum and non-immune serum alone were 94 483 ± 2398 and 156 841 ± 2349 cpm/well, respectively. The assay was performed in quadruplicate.

**The DNA synthesis inhibition (15%) was significantly less when compared with that (~40%) in cells treated with TGF-β_1 alone or TGF-β_1 + insulin or anti-α5β1 integrin serum (Student's *t* test, $P < 0.001$).

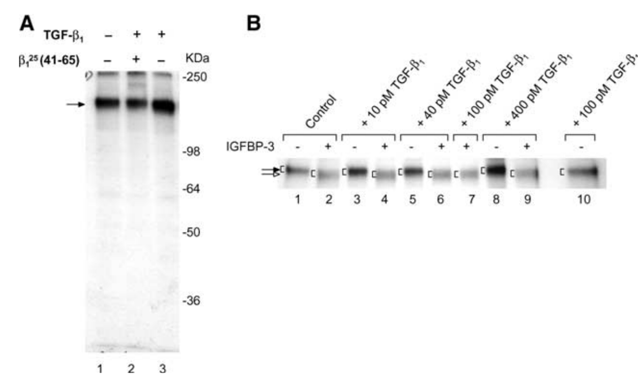

Fig. 2. TGF-β_1-induced phosphorylation of IRS-2 in Mv1Lu cells (A) and DR26 cells (B). Mv1Lu cells (A) metabolically labeled with ^{32}P-orthophosphate for 2 h were treated with TGF-β_1 (100 pM) in the presence or absence of $\beta_1{}^{25}$(41–65), a TGF-β peptide antagonist (20 μM). DR26 cells (B) metabolically labeled with ^{32}P-orthophosphate for 2 h were treated with or without IGFBP-3 (1 μg/ml) in the presence of increasing concentrations (0, 10, 40, 100 and 400 pM) of TGF-β_1 as indicated. After 2 h at 37 °C, equal amounts of protein from the cell lysates of ^{32}P-labeled cells were immunoprecipitated with anti-LRP-1 serum. The immunoprecipitates were then analyzed by 7.5% SDS–PAGE and autoradiography (quantitated with a PhosphoImager). The arrow indicates the location of ^{32}P-labeled IRS-2 (A). The brace indicates the location of ^{32}P-labeled IRS-2 (B). The closed and open arrows indicate the locations of the slow or faster migrating forms of ^{32}P-labeled IRS-2 (B).

results suggest that the TGF-β_1-stimulated phosphorylation of IRS-2 may be involved in TGF-β_1-induced growth inhibition because the TGF-β_1-stimulated phosphorylation of IRS-2 is also blocked by $\beta_1{}^{25}$(41–65), a TGF-β peptide antagonist which is known to reverse TGF-β growth inhibition by blocking TGF-β binding to TGF-β receptors [7,23,26,27].

3.3. IRS proteins are involved in growth inhibition by TGF-β_1

Since insulin is capable of partially reversing growth inhibition by TGF-β_1 in the presence of a cyclic RGD peptide or anti-α5β1 integrin serum and since IRS proteins are required for TβR-V-mediated growth inhibition by IGFBP-3 [26], we hypothesized that IRS proteins are also involved in TGF-β_1-induced growth inhibition. To test this hypothesis, we examined the effect of TGF-β_1 on cell growth (as determined by measurement of DNA synthesis) of 32D cells stably transfected with IRS cDNAs, IR cDNA, or vector only. 32D cells are murine myeloid cells which do not express IRS proteins [28,33]. They express very low levels of the IR and high levels of TβR-V ([33], unpublished results). They also respond weakly to growth inhibition by TGF-β_1 but have a functional TβR-I/TβR-II heterocomplex-mediated signaling cascade as determined by measurement of TGF-β_1-induced transcriptional activation of plasminogen activator inhibitor-1 ([26], data not shown). For these reasons, the 32D cell system should be appropriate for defining the roles of IRS proteins in TGF-β_1 growth inhibition. As shown in Fig. 3, TGF-β_1 inhibited DNA synthesis of 32D cells transfected with vector only minimally (Fig. 3A). However, 32D cells expressing either IRS-1 or IRS-2 (32D/IRS-1 or 32D/IR/IRS-2 cells) exhibited a robust growth inhibitory response to TGF-β_1 (Fig. 3A and B). Insulin was capable of partially reversing growth inhibition by TGF-β_1 in 32D cells stably expressing the IR and IRS-2 (32D/IR/IRS-2 cells) (Fig. 3B). These results suggest that IRS proteins are involved in growth inhibition by TGF-β_1.

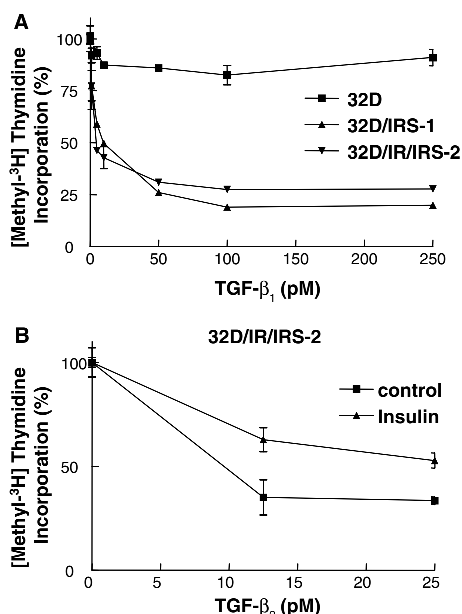

Fig. 3. Growth inhibition induced by TGF-β_1 in 32D/IRS-1 and 32D/IR/IRS-2 cells but not in 32D cells (A) and insulin reversal of growth inhibition by TGF-β_1 in 32D/IR/IRS-2 cells (B). Cells as indicated were treated with increasing concentrations of TGF-β_1 in the absence (A) or presence (B) of insulin (10 nM). After 18 h at 37 °C, DNA synthesis was determined by measuring [methyl-^{3}H] thymidine incorporation into cellular DNA. The [methyl-^{3}H] thymidine incorporation in cells treated without TGF-β_1 ($824\,100 \pm 18\,400$, $384\,910 \pm 10\,290$ and $58\,051 \pm 3598$ cpm/well for 32D, 32D/IRS-1 and 32D/IR/IRS-2 cells, respectively) was taken as 100% of DNA synthesis. Each data point is the mean ± S.D. of quadruplicate determinations.

4. Discussion

TGF-β is the most potent known growth inhibitor for epithelial cells [34]. Very few growth factors antagonize the growth inhibition activity of TGF-β [1–5]. Here, we demonstrate that insulin partially reverses growth inhibition by TGF-β_1 in the presence of α5β1 integrin antagonists in Mv1Lu cells. Since fibronectin, a prominent ligand of α5β1 integrin, has been shown to attenuate tyrosine phosphorylation of IRS proteins stimulated by insulin and since TGF-β stimulates the expression of fibronectin in the same cells [30], this result suggests that: (1) the TGF-β_1-stimulated expression of fibronectin (which is mainly mediated by the TβR-I/TβR-II/Smad2/3/4 signaling cascade) renders Mv1Lu cells insensitive to insulin-mediated reversal of TGF-β_1 growth inhibition, and (2) the TβR-I/TβR-II/Smad2/3/4 signaling cascade is capable of regulating TβR-V-mediated signaling (which is required for growth inhibition by TGF-β_1) by positive feedback (via induction of fibronectin expression). This suggestion is supported by the observation that insulin partially reverses growth inhibition by TGF-β_1 in 32D/IR/IRS-2 cells. These 32D cells do not express detectable α5β1 integrin and do not respond to fibronectin attenuation of insulin-stimulated tyrosine phosphorylation of IRS-2 as other cell types do ([30], data not shown).

Two major signaling pathways are involved in the events which follow insulin activation of the IR and the subsequent activated IR-mediated tyrosine phosphorylation of IRS proteins. These are the PI 3-kinase and MAP kinase pathways [35,36]. These pathways do not appear to be involved in insulin-mediated reversal of growth inhibition by either IGFBP-3 [26] or TGF-β_1 as evidenced by: (1) Insulin is capable of reversing growth inhibition by IGFBP-3 in Mv1Lu cells treated with PI 3-kinase inhibitors (Wortmannin and LY-294002) [26,32]. (2) Growth factors such as EGF and FGF do not reverse growth inhibition by either IGFBP-3 or TGF-β_1 [26,32]. These growth factors are potent stimulators of the MAP kinase pathway in Mv1Lu cells. IRS proteins are likely to be involved in growth inhibition by IGFBP-3 and TGF-β_1. We previously showed that IRS proteins are required for growth inhibition by IGFBP-3 [26]. Here, we demonstrate that IRS proteins are also required for growth inhibition by TGF-β_1. This is evidenced by: (1) Insulin partially reverses growth inhibition by TGF-β_1 in the presence of α5β1 integrin antagonists (Cyclo GRGDPSA and anti-α5β1 integrin serum), (2) stable transfection of 32D murine myeloid cells (which lack expression of IRSs) with IRS-1 or IRS-2 cDNA confers sensitivity to TGF-β growth inhibition, and (3) insulin partially reverses TGF-β-induced growth inhibition in 32D cells stably expressing IRS-2 and IR.

We recently found that insulin reverses IGFBP-3 growth inhibition by inducing serine-specific dephosphorylation of IRS proteins in Mv1Lu cells and mutant cells derived from

Mv1Lu cells (DR26 cells) [26]. Here, we demonstrate that TGF-β_1 induces serine-specific phosphorylation of IRS-2 and that this is not mediated by TβR-II because TGF-β_1 is capable of inducing such phosphorylation in DR26 cells which do not express functional TβR-II but do express TβR-V and TβR-I. TβR-I has been shown to be incapable of binding ligands in the absence of TβR-II [11,12]. The ability of TβR-V to mediate such distinct activities (dephosphorylation and phosphorylation) upon IGFBP-3 and TGF-β_1 binding (in Mv1Lu and DR26 cells) is intriguing. TβR-V may form different complexes with plasma membrane proteins (e.g., co-receptors) and cytoplasmic proteins (e.g., protein kinases and phosphatases), which respond differently to IGFBP-3 or TGF-β_1 binding as measured by the downstream effects on the phosphorylation status of IRS proteins. There are three lines of evidence to support this possibility: (1) IGFBP-3 and TGF-β_1 bind to distinct sites in the TβR-V molecules (unpublished results). (2) LRP-1, which is identical to TβR-V, has been shown to be a component of signaling complexes containing protein phosphatases or kinases [37–42]. (3) Most of the TβR-V in Mv1Lu cells is not required for the growth inhibitory response to IGFBP-3 and TGF-β_1 [23]. Mutagenized Mv1Lu cells selected for reduced expression ($\leqslant 5\%$) of the TβR-V still respond to growth inhibition by IGFBP-3 and TGF-β_1 although the growth inhibitory response in these Mv1Lu mutant cells is attenuated [23]. This evidence supports the view that TβR-V exists in heterogeneous populations and that only a small fraction of cell-surface TβR-V is required to mediate the growth inhibitory response.

The finding of cross talk (via IRS proteins) between the insulin-induced signaling and TGF-β-induced (TβR-V/LRP-1-mediated) growth inhibitory signaling cascades has potential clinical implications. Insulin or insulin signaling defects may up-regulate the TGF-β activity generated in wounds, resulting in the attenuation of wound re-epithelialization and healing [43], which is a common clinical problem observed particularly often in diabetic patients.

Acknowledgements: We thank Dr. Frank E. Johnson and Dr. William S. Sly for critical review of the manuscript and John McAlpin for typing the manuscript. We also thank Dr. Martin G. Myers, Jr., Research Division, Joslin Diabetes Center, Boston, for providing 32D, 32D/IRS-1 and 32D/IR/IRS-2 cells.

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