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The estrogen receptor α:insulin receptor substrate 1 complex in breast cancer: structure–function relationships

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Background: Insulin receptor substrate 1 (IRS-1) is a signaling molecule that exerts a key role in mediating cross talk between estrogen receptor α (ERα) and insulin-like growth factor 1 (IGF-1) in breast cancer cells. Previously, we demonstrated that a fraction of IRS-1 binds ERα, translocates to the nucleus, and modulates ERα-dependent transcription at estrogen response elements (ERE). Here, we studied structure–function relationships of the ERα:IRS-1 complex under IGF-1 and/or estradiol (E_2) stimulation.
Materials and methods: ERα and IRS-1 deletion mutants were used to analyze structural and functional ERα/IRS-1 interactions. IRS-1 binding to ERE and IRS-1 role in ERα-dependent ERE transcription was examined by chromatin immunoprecipitation and gene reporter analysis, respectively. The requirement for IRS-1 in ERα function was tested with RNAi technology.
Results: Nuclear translocation of IRS-1 was induced by E_2, IGF-1, and a combination of both stimuli. ERα/IRS-1 binding was direct and involved the activation function-1 (AF-1)/DNA binding domain (DBD) region of ERα and two discrete regions of IRS-1 (the N-terminal pleckstrin homology domain and a region within the C-terminus). IRS-1 knock down abrogated IGF-1-dependent transcriptional activity of unliganded ERα, but induced the activity of liganded ERα.
Conclusions: ERα/IRS-1 interactions are direct and involve the ERα AF-1/DBD domain and IRS-1 domains mapping within N- and C-terminus. IRS-1 may act as a repressor of liganded ERα and coactivator of unliganded ERα.
Key words: estrogen receptor alpha (ERa), Insulin receptor substrate 1 (IRS-1), breast cancer

introduction

Insulin-like growth factor-1 (IGF-1) and 17-β-estradiol (E_2) have been shown to act in synergy, stimulating breast cancer cell growth and survival [1, 2]. The functional interactions between E_2 and IGF-1 signaling systems involve several transcriptional and posttranscriptional mechanisms. For example, IGF-1 can affect estrogen receptor α (ERα) action by enhancing its expression and potentiating its transcriptional activity in a ligand-independent manner [3–7]. On the other hand, E_2 can enhance IGF-1 signaling by upregulating the expression of IGF-1 [8], IGF-1 receptor [9], and some IGF-1 binding proteins [10]; ERα also stimulates transcription and enhances stability of insulin receptor substrate 1 (IRS-1), a major IGF-1 signaling molecule [11–13].

IRS-1 is a 130–180 kDa docking protein containing two conserved domains within the N-terminal portion. The PH (pleckstrin homology) domain mediates interactions with phospholipids and proteins containing acidic motifs. The phosphotyrosine-binding (PTB) domain couples IRS-1 with the phosphorylated IGF-1 receptor. The IRS-1 C-terminus contains several serine and tyrosine residues that can modulate its activity. The major intracellular pathways stemming from IRS-1 are activated upon its tyrosine phosphorylation and subsequent recruitment of downstream signaling molecules through Src homology domain-type interactions [14, 15].

Numerous studies have shown that in breast cancer cells, IRS-1 signaling regulates cell proliferation, survival, and drug resistance. IRS-1 is also a key molecule sustaining efficient E_2/IGF-1 cross talk [11, 16]. Recently, we described that in addition to its function as a signaling molecule, IRS-1 might affect nuclear processes. Specifically, IRS-1 can be found in the nucleus in breast cancer cells where it can interact with ERα. In breast tumors, nuclear colocalization of IRS-1 and ERα negatively correlated with tumor grade, size, mitotic index, and lymph node involvement in ductal breast cancer tissues [17]. The function of nuclear IRS-1 in the regulation of steroid receptor function is not well defined; our data

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indicated that nuclear IRS-1 can act as transcriptional regulator of liganded ERα at estrogen response elements (ERE) in DNA [18].

In this study, we examined how E_2, IGF-1, and the combination of both factors regulate IRS-1 nuclear translocation, its binding with ERα, and its effects on ERα-mediated transcription. Furthermore, using different deletion mutants of IRS-1 and ERα, we characterized structure–function relationships in the ERα:IRS-1 complex.

methods and results

E_2 and IGF-1 modulate nuclear translocation of IRS-1 and its recruitment to ERE sites

Previously, we reported that IRS-1 colocalizes and coprecipitates with ERα in ER-positive MCF-7 cells and that a fraction of IRS-1 can be translocated to the nucleus together with liganded ERα [18]. Here, we asked whether IRS-1 could be transported to the nucleus in response to IGF-1 or IGF-1 plus E_2 treatments. Under serum-free medium conditions, IRS-1 was present mainly in the cytoplasm. The addition of E_2 for 1 or 4 h significantly increased nuclear abundance of IRS-1 and reduced its cytoplasmic content (Figure 1A). Similar effects were seen with the combination of E_2 and IGF-1. IGF-1 alone minimally increased IRS-1 nuclear translocation at 4 h (Figure 1A).

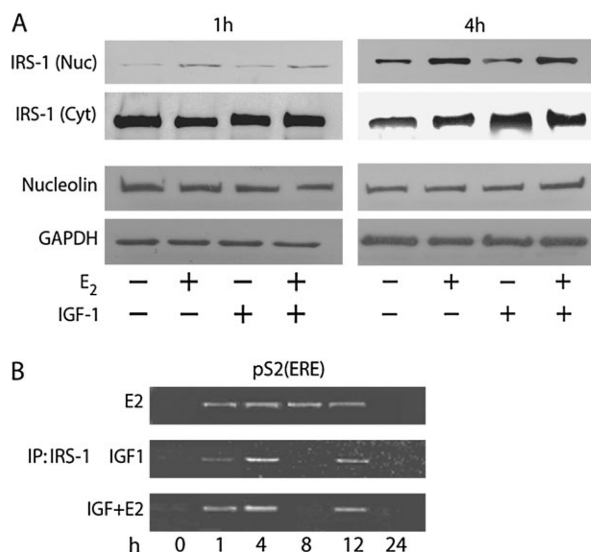

Figure 1. Insulin receptor substrate 1 (IRS-1) associates with the pS2 ERE motif in insulin-like growth factor 1 (IGF-1) and estradiol (E_2)-treated MCF-7 cells. (A) MCF-7 cells synchronized in serum-free medium were left untreated or were treated with 10 nM E_2, and/or 20 ng/ml IGF-1 for 24 h. The abundance and localization of IRS-1 was analyzed by western blotting using 50 μg of cytoplasmic and nuclear proteins. The purity of subcellular protein fractions was monitored by probing for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and nucleolin, serving as cytoplasmic and nuclear protein markers, respectively. The antibodies (Abs) used were described previously [18]. (B) Chromatin immunoprecipitation assays were carried out as described previously [18]. Briefly, MCF-7 cells were treated with 10 nM E_2 and/or 20 ng/ml IGF-1, for 1, 4, 8, 12, and 24 h. Next, the cells were cross-linked with paraformaldehyde and chromatin–protein complexes were immunoprecipitated with a specific IRS-1 Ab. The presence of pS2 ERE in the resulting immunoprecipitates was analyzed by PCR [18].

Next, we analyzed the association of IRS-1 with ERE sequences within the pS2 gene promoter (Figure 1B). We found that E_2 stimulated IRS-1 loading on pS2 ERE from 1 h to 12 h, reaching the maximum at 4 h, which was concomitant with the increased nuclear translocation of IRS-1 (Figure 1A and B). On the other hand, IGF-1 stimulation produced two peaks in the IRS-1 binding on pS2 ERE promoter, at 4 h and 12 h. The addition of E_2 significantly improved IGF-1-induced recruitment of IRS-1 on pS2 ERE at 4 h. Interestingly, at 8 h, IRS-1 was loaded on pS2 in response to E_2 but not under IGF-1 or E_2 plus IGF-1, indicating involvement of IRS-1 in IGF-1 signaling at these time points.

characteristics of the ERα:IRS-1 complex

To characterize the region of IRS-1 responsible for ERα binding under different stimuli, we employed IRS-1 truncation mutants (depicted in Figure 2A) [15]. The glutathione S-transferase fusion protein incorporating IRS-1 (GST-IRS-1) mutants were incubated with 100 μg of either cytoplasmic or nuclear proteins obtained from MCF-7 cells stimulated with E_2 and/or IGF-1, or left untreated. In unstimulated cells, the strongest ERα binding mapped within the first 300 amino acids of IRS-1 (M1); a less efficient binding was also detected with the last 500 amino acids corresponding to the mutants M4 and M5 (Figure 2B). The IRS-1 M1 region contains the PH domain and a portion of the PTB domain [14, 19]. The absence of binding with the M2 mutant, containing 97 amino acids of the PTB domain, indicates that this domain is not involved in ERα/IRS-1 interactions (Figure 2B). ERα binding to IRS-1 M1, M4, and M5 domains occurred under all stimulation conditions (Figure 2C), indicating that these interactions are not affected by conformational changes and/or phosphorylation induced by stimulation with IGF-1 and/or E_2. The question whether ERα/IRS-1 binding is direct or requires other proteins was addressed with by incubating GST-IRS-1 mutants with a synthetic ERα protein. The results demonstrated efficient ERα binding to M1, and to a lesser extent to M4 and M5 *in vitro* (Figure 2D), indicating that ERα directly interacts with IRS-1.

To map ERα regions involved in IRS-1 binding, we first used ERα deletion mutants lacking the activation function-1 (AF-1)/DNA binding domain (DBD) or activation function-2 (AF-2) domain [20] (Figure 3). Using GST pull-down assays, we demonstrated that IRS-1 binds to AF-1/DBD, but not to AF-2 (Figure 3A and B). Interestingly, stimulation with E_2, IGF-1, or both increased AF-1/DBD/IRS-1 binding in the nucleus, decreasing their cytoplasmic interactions (Figure 3A). A more detailed mapping of ERα:IRS-1 interfaces was done using additional GST-ERα truncation mutants (Figure 3C). Specifically, we tested Δ1 and Δ2 mutants that include the AF-1 domain, Δ3 that includes a part of the AF-1 domain and the entire DBD, Δ4 that covers the AF-2 domain and a part of DBD, and Δ5 that includes a major portion of the AF-2 domain [21] (Figure 3C). The results confirmed that IRS-1 binds to the AF-1/DBD domain of ERα (Figure 3D).

effects of IRS-1 knock down on ERα-mediated transcription at pS2 ERE

To investigate functional interactions between IRS-1 and the ERα AF-1 domain, we employed a luciferase transcription reporter assays (Figure 4). HeLa cells (ERα negative, IRS-1 positive) were transiently cotransfected with the ERE-responsive luciferase reporter plasmid and a plasmid encoding ERα (pSG5-HeG0, Figure 4B), ERα with C-terminal truncation (encoding ERα AF-1/DBD domain pSG5-HE15, Figure 4C), ERα with N-terminal truncation (encoding ERα AF-2/DBD domain, pSG5-HE19, Figure 4D), or an empty vector (pSG5, Figure 4A) [22]. To test the role of IRS-1 in ERα-mediated transcription, IRS-1 levels were downregulated by 70% using anti-IRS-1 siRNA, as described before [23]. We observed a significant increase of E_2-induced ERE transcription in the absence of IRS-1 (Figure 4B). In contrast, downregulation of IRS-1 reduced ERE

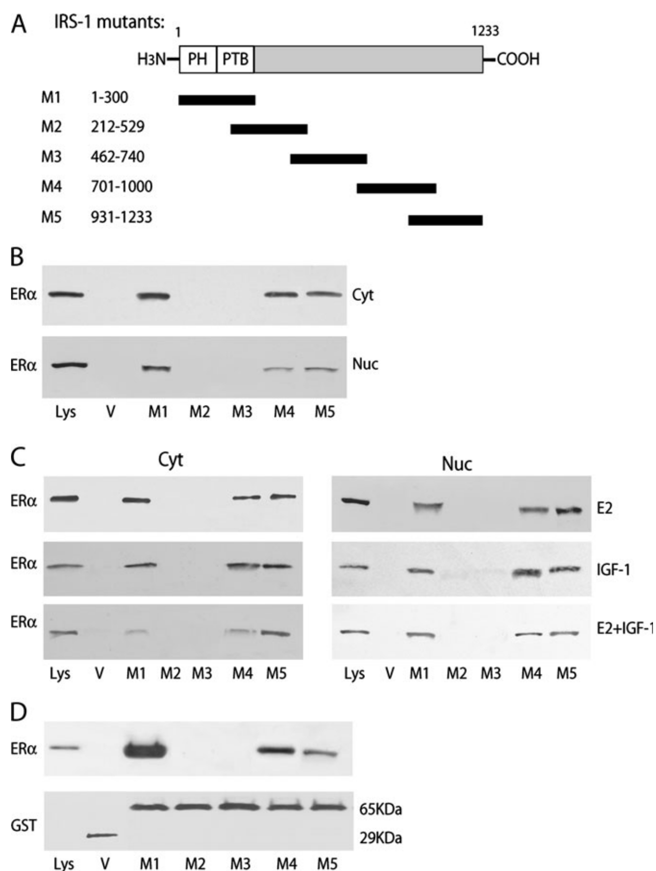

Figure 2. Insulin receptor substrate 1 (IRS-1) domains involved in estrogen receptor α (ERα) binding; influence of estradiol (E_2) and insulin-like growth factor 1 (IGF-I) treatment. (A) We expressed and purified GST-IRS-1 fusion proteins according to previously described protocol [15]. MCF-7 cells synchronized in serum-free medium were left untreated (B) or treated with 10 nM E_2, or 20 ng/ml of IGF-1 or both (C) for 24 h and then lysed. Hundred microgram of cytoplasmic or nuclear proteins were precipitated with 10 μg of GST (V) or appropriate GST-IRS-1 truncation mutants coupled to glutathione-Sepharose. The bound proteins were eluted and analyzed by western blotting (WB) with anti-ERα monoclonal antibody (mAb) (Santa Cruz Biotechnology Santa Cruz, CA). Twenty microgram of total lysates were loaded as control (Lysate [Lys]). Three mutants, amino acids 1–300 (pleckstrin homology/ phosphotyrosine-binding domain), amino acids 701–1000, and amino acids 931–1233, are positive for the interaction with ERα. All other mutants and GST alone are negative. (D) Ten nanogram of ERα pure protein (Promega Madison, WI) was precipitated with 10 μg of GST or GST-IRS-1 truncation mutants coupled to glutathione-Sepharose. Precipitates were analyzed by WB with anti-ERα mAb and anti-GST antibody (Santa Cruz Biotechnology).

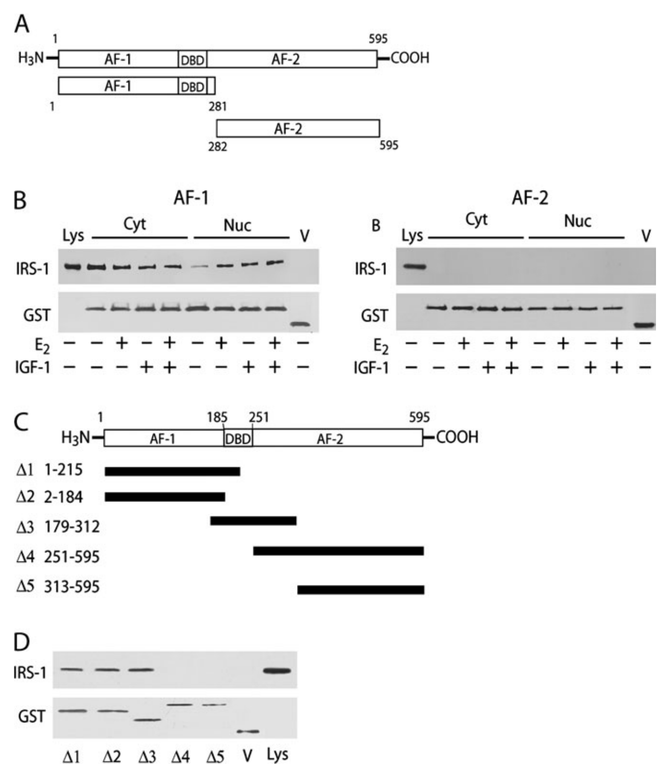

Figure 3. Estrogen receptor α (ERα) domains involved in insulin receptor substrate 1 (IRS-1) binding. (A) We expressed and purified the GST-ERα fusion proteins with activation function-1/DNA binding domain or activation function-2 deletions, as described previously [15]. (B) MCF-7 were left untreated or treated with 10 nM estradiol, and/or 20 ng/ml of insulin-like growth factor 1 for 24 h. Hundred microgram of cytoplasmic or nuclear proteins were precipitated with 10 μg of GST (V) or different GST-ERα truncation mutants coupled to glutathione-Sepharose. IRS-1 and GST content in precipitates were determined by western blotting (WB). Twenty microgram of total lysates were loaded as control [Lys]). (C) A more detailed mapping was carried out with shorter GST-ERα fragments. (D) Cell lysates were precipitated with 10 μg of GST (V) or different GST-ERα truncation mutants coupled to glutathione-Sepharose. IRS-1 and GST content in precipitates were determined by WB.

transcription in IGF-1 and IGF-1 plus E_2-treated cells (Figure 4B). IRS-I knock down did not significantly influence ERE-mediated transcription in HeLa cells expressing the AF-2/DBD region of ERα (Figure 4D), while a significant decrease of ERα transactivation was observed in cells expressing the AF-1/DBD region in response to IGF-1 stimulation.

discussion

ERα/IGF-1 cross talk is known to influence breast cancer cell proliferation, survival, transformation, migration, and invasion [2, 24, 25]. IRS-1 is a major substrate of the IGF-1 receptor and a crucial molecule mediating ERα/IGF-1 interactions [1, 2, 14]. In breast cancer, IRS-1 overexpression has been associated with the development of the transformed phenotype, hormone independence, and drug resistance [2]. These effects have been attributed to increased IRS-1 tyrosine phosphorylation and potentiation of its signaling through the antiapoptotic Akt pathway [2, 25]. In addition to its conventional role as signal transducing molecule, IRS-1 has been found in the nuclear compartment in several cell types [15, 17, 18, 26, 27]. Recently, we demonstrated that nuclear IRS-1 is present in ERα-positive breast tumors and cell lines. In cellular systems, we found that IRS-1 can interact with ERα and influence the activity of liganded ERα [18]. Here, we characterized IRS-1 and ERα domains that are involved in functional interactions between these molecules and we studied how IGF-1 can influence nuclear localization of IRS-1 and its recruitment on ERE-containing promoters in the presence of liganded or unliganded ERα.

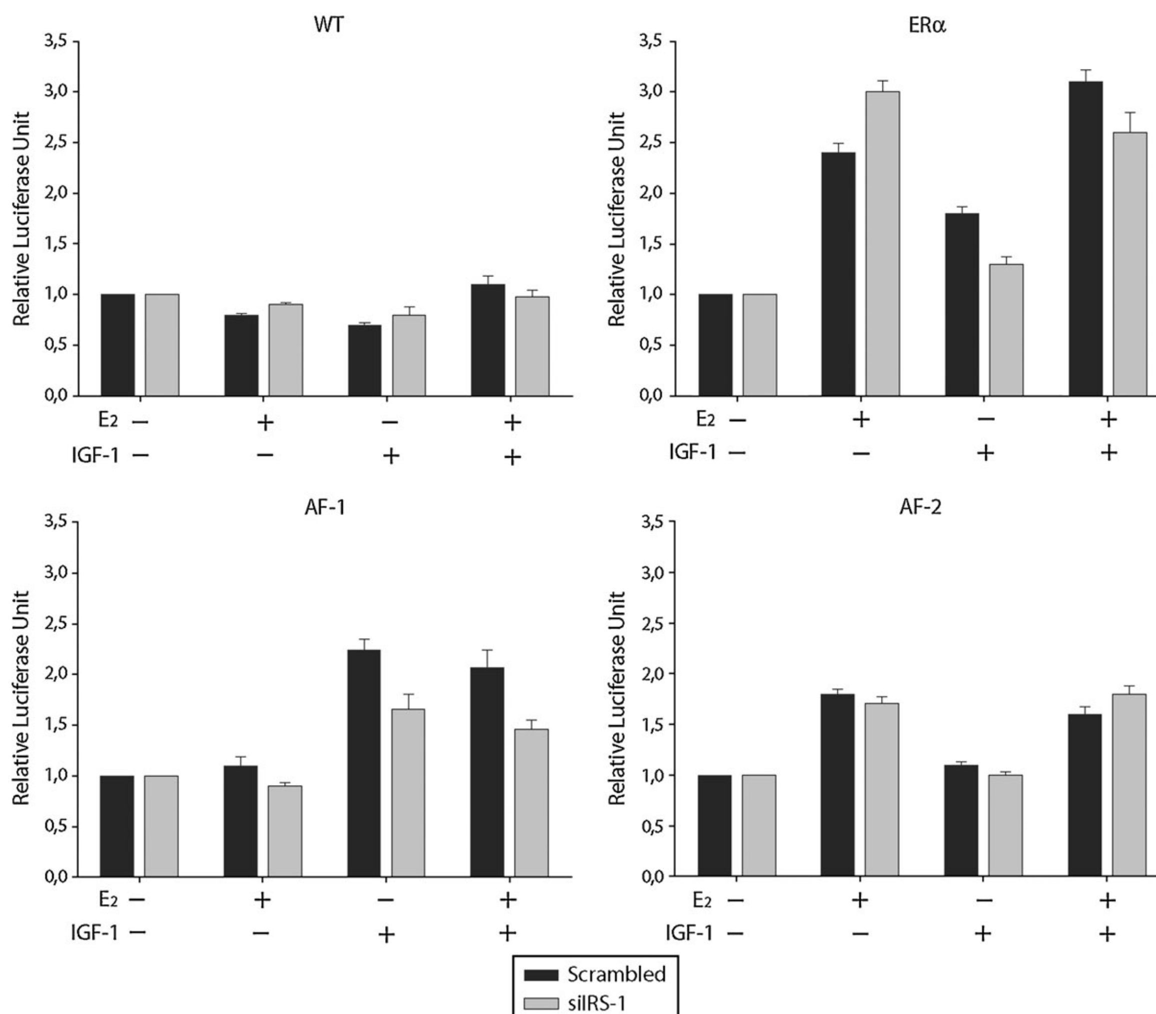

Figure 4. Insulin receptor substrate 1 is a transcriptional coregulator of estrogen receptor α. The experiments were carried out using HeLa cells that are ERα negative and IRS-1 positive. All transfection mixtures contained the reporter plasmid, ERE-Luc, encoding the firefly luciferase complementary DNA under the control of the TK promoter and three estrogen response element sequences and, as internal control, the plasmid pRL-Tk (Promega) encoding Renilla reniformis luciferase. The cocktail was cotransfected with either the empty vector pSG5 (A), pSG5-HeG0 encoding ERα (B), pSG5-HE15, and pSG5-HE19, code for a C-terminal truncated receptor (activation function-1/DNA binding domain (DBD), amino acids 1–281) (C) and for the N-terminal truncated receptor (activation function-2/DBD, amino acids 179–575) (D), respectively. The luciferase activity was measured using Dual luciferase assay System (Promega Madison, WI). IRS-1 knock down was obtained by transfecting cells with pSilencer-IRS-1 plasmid (shIRS1) [23] or with a control scrambled shRNA (Scrambled). Transfections and luciferase assays were carried out as described previously [18]. The results represent mean ± standard deviation of five independent experiments.

Our results indicated that the interaction between IRS-1 and ERα does not require intermediating proteins as it can occur *in vitro* between GST-IRS-1 mutants and synthetic ERα. Two binding sites for ERα were mapped on IRS-1. One site mapped within the N-terminal portion of IRS-1 containing the PH domain, while the second localized within the C-terminus of IRS-1 [14]. These results are consistent with previously published observations that nuclear IRS-1 can interact with other proteins (e.g. the T antigen of JCV virus) via the PH domain [15]. The binding site for IRS-1 on ERα was mapped in the AF-1/DBD domain that contains several serine residues responsible of ligand-independent transactivation of ERα [2, 5, 28]. However, because the ERα/IRS-1 complex can bind to ERE under E_2, which must engage an unoccupied DBD domain, we speculate that ERα binding to IRS-1 is mediated mostly by AF-1.

Nuclear translocation of IRS-1 and its interaction with ERE could be induced by both E_2 and IGF-1, but with different dynamics and efficiency. E_2 activates continuous presence of IRS-1 on ERE, while IGF-1 stimulates intermittent IRS-1 interaction with these sites. Notably, IRS-1 recruitment to ERE in response to E_2 and IGF-1 resembles that of liganded or unliganded ERα, respectively [29], indicating that IRS-1 and ERα bind ERE motifs as one complex. The differential recruitment of the ERα:IRS-1 complex could be explained by the nature of ERα activation in response to E_2 or IGF-1. In particular, E_2 directly activates ERα by binding to the AF-2 domain [30]. Instead, activation of ERα by IGF-1 is indirect and mediated by Erk1/2 and Akt kinases that phosphorylate ERα AF-1 domain on serine residues 118 and 167, respectively [1, 5, 28, 31, 32]. Notably, the recruitment of

IRS-1 on ERE site in response to a combination of IGF-1 and E_2 was greater than that seen with either IGF-1 or E_2 alone, confirming synergistic effects of both mitogens on ERα.

Finally, we investigated the relevance of IRS-1/ERα interaction in ERα-dependent transcription in response to E_2 and/or IGF-1 stimulation. Using IRS-1 RNAi technology, we confirmed that IRS-1 might act as a repressor of liganded ERα on ERE [18]. It is worth noting that the effects of IRS-1 knock down were not noticeable in cells expressing the AF-1 or the AF-2 truncated mutants of ERα. This is in agreement with IRS-1 function since the absence of IRS-1 reduces the recruitment of protein kinases that phosphorylate serine residues within the AF-1 domain inducing ligand-independent activation of ERα [16, 33]. On the other hand, our results indicated that IRS-1 might be a coactivator of unliganded (IGF-1 transactivated) ERα. The negative effects of IRS-1 towards liganded ERα were abrogated under combined E_2 plus IGF-1 treatment, indicating that cooperation of both stimuli might be optimal for ERα transcriptional response. In conclusion, our data indicate that IRS-1 interacts directly with ERα in the nucleus of breast cancer cells and plays a key role in the regulation of balanced transcription of liganded and unliganded ERα.

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references

1. Sisci D, Surmacz E. Crosstalk between IGF signaling and steroid hormone receptors in breast cancer. Curr Pharm Des 2007; 13: 705–717.
2. Surmacz E. Function of the IGF-I receptor in breast cancer. J Mammary Gland Biol Neoplasia 2000; 5: 95–105.
3. Lange CA. Making sense of cross-talk between steroid hormone receptors and intracellular signaling pathways: who will have the last word? Mol Endocrinol 2004; 18: 269–278.
4. Weigel NL, Zhang Y. Ligand-independent activation of steroid hormone receptors. J Mol Med 1998; 76: 469–479.
5. Lannigan DA. Estrogen receptor phosphorylation. Steroids 2003; 68: 1–9.
6. Edwards DP, Weigel NL, Nordeen SK, Beck CA. Modulators of cellular protein phosphorylation alter the trans-activation function of human progesterone receptor and the biological activity of progesterone antagonists. Breast Cancer Res Treat 1993; 27: 41–56.
7. Shupnik MA. Crosstalk between steroid receptors and the c-Src-receptor tyrosine kinase pathways: implications for cell proliferation. Oncogene 2004; 23: 7979–7989.
8. Umayahara Y, Kawamori R, Watada H et al. Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. J Biol Chem 1994; 269: 16433–16442.
9. Stewart AJ, Johnson MD, May FE, Westley BR. Role of insulin-like growth factors and the type I insulin-like growth factor receptor in the estrogen-stimulated proliferation of human breast cancer cells. J Biol Chem 1990; 265: 21172–21178.
10. Qin C, Singh P, Safe S. Transcriptional activation of insulin-like growth factor-binding protein-4 by 17beta-estradiol in MCF-7 cells: role of estrogen receptor-Sp1 complexes. Endocrinology 1999; 140: 2501–2508.
11. Salerno M, Sisci D, Mauro L et al. Insulin receptor substrate 1 is a target for the pure antiestrogen ICI 182,780 in breast cancer cells. Int J Cancer 1999; 81: 299–304.
12. Molloy CA, May FE, Westley BR. Insulin receptor substrate-1 expression is regulated by estrogen in the MCF-7 human breast cancer cell line. J Biol Chem 2000; 275: 12565–12571.
13. Lee AV, Jackson JG, Gooch JL et al. Enhancement of insulin-like growth factor signaling in human breast cancer: estrogen regulation of insulin receptor substrate-1 expression in vitro and in vivo. Mol Endocrinol 1999; 13: 787–796.
14. White MF. The insulin signalling system and the IRS proteins. Diabetologia 1997; 40 (Suppl 2): S2–S17.
15. Lassak A, Del Valle L, Peruzzi F et al. Insulin receptor substrate 1 translocation to the nucleus by the human JC virus T-antigen. J Biol Chem 2002; 277: 17231–17238.
16. Ma ZQ, Santagati S, Patrone C et al. Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3. Mol Endocrinol 1994; 8: 910–918.
17. Sisci D, Morelli C, Garofalo C et al. Expression of nuclear insulin receptor substrate 1 (IRS-1) in breast cancer. J Clin Pathol 2006; [Epub ahead of print].
18. Morelli C, Garofalo C, Sisci D et al. Nuclear insulin receptor substrate 1 interacts with estrogen receptor alpha at ERE promoters. Oncogene 2004; 23: 7517–7526.
19. Myers MG Jr., Sun XJ, White MF. The IRS-1 signaling system. Trends Biochem Sci 1994; 19: 289–293.
20. Kumar V, Green S, Stack G et al. Functional domains of the human estrogen receptor. Cell 1987; 51: 941–951.
21. Platet N, Cunat S, Chalbos D et al. Unliganded and liganded estrogen receptors protect against cancer invasion via different mechanisms. Mol Endocrinol 2000; 14: 999–1009.
22. Sisci D, Aquila S, Middea E et al. Fibronectin and type IV collagen activate ERalpha AF-1 by c-Src pathway: effect on breast cancer cell motility. Oncogene 2004; 23: 8920–8930.
23. Cesarone G, Garofalo C, Abrams MT et al. RNAi-mediated silencing of insulin receptor substrate 1 (IRS-1) enhances tamoxifen-induced cell death in MCF-7 breast cancer cells. J Cell Biochem 2006; 98: 440–450.
24. Surmacz E, Bartucci M. Role of estrogen receptor alpha in modulating IGF-I receptor signaling and function in breast cancer. J Exp Clin Cancer Res 2004; 23: 385–394.
25. Sachdev D, Yee D. The IGF system and breast cancer. Endocr Relat Cancer 2001; 8: 197–209.
26. Prisco M, Santini F, Baffa R et al. Nuclear translocation of insulin receptor substrate-1 by the simian virus 40 T antigen and the activated type 1 insulin-like growth factor receptor. J Biol Chem 2002; 277: 32078–32085.
27. Sun H, Tu X, Prisco M et al. Insulin-like growth factor I receptor signaling and nuclear translocation of insulin receptor substrates 1 and 2. Mol Endocrinol 2003; 17: 472–486.
28. Martin MB, Franke TF, Stoica GE et al. A role for Akt in mediating the estrogenic functions of epidermal growth factor and insulin-like growth factor I. Endocrinology 2000; 141: 4503–4511.
29. Cascio S, Bartella V, Garofalo C et al. Insulin-like growth factor 1 differentially regulates estrogen receptor-dependent transcription at estrogen response element and AP-1 sites in breast cancer cells. J Biol Chem 2007; 282: 3498–3506.
30. Wegele H, Muller L, Buchner J. Hsp70 and Hsp90–a relay team for protein folding. Rev Physiol Biochem Pharmacol 2004; 151: 1–44.
31. Kato S, Endoh H, Masuhiro Y et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 1995; 270: 1491–1494.
32. Bunone G, Briand PA, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. EMBO J 1996; 15: 2174–2183.
33. Lee AV, Weng CN, Jackson JG, Yee D. Activation of estrogen receptor-mediated gene transcription by IGF-I in human breast cancer cells. J Endocrinol 1997; 152: 39–47.