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IGF-I regulates caveolin 1 and IRS1 interaction in caveolae

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Abstract

Caveolae are hot spots in IGF-I signalling as suggested by the facts that IGF-I receptors localize in caveolae, directly interact with and tyrosine phosphorylate caveolin 1, the major caveolar protein. Also a number of IGF-IR substrates reside in caveolae, supporting a role of these organelles in the regulation of IGF-I action. Recently, we have demonstrated that IGF-I could specifically regulate Shc phosphorylation in caveolae. Here we show that also IRS1 localizes in this region where it is tyrosine phosphorylated in the presence of IGF-I. Moreover, IRS1 co-immunoprecipitates with caveolin 1 and the specific phosphocaveolin 1–IRS1 interaction is increased by IGF-I.

Keywords: IGF-I receptor; IRS1; Caveolin 1; Tyrosine phosphorylation

Caveolae are plasma membrane regions derived from lipid rafts by oligomerization of caveolin proteins. Caveolae regulate several cellular processes and a specific role of these organelles as centers of signalling pathways has been proposed [1]. Caveolins derive from three different genes and show a specific cellular expression, caveolin 1 and 2 being present in most cell types, while caveolin 3 is muscle specific [2]. Many functions of caveolae are mediated by caveolin 1. Caveolin 1 directly interacts with a number of signalling proteins at the level of its scaffolding domain [3]. Insulin receptor (IR) and IGF-I receptor (IGF-IR) are highly enriched in caveolae [4–6] and a role of caveolae in the IGF-I signalling in multiple myeloma cells [7] as well as in the differentiation of 3T3-L1 fibroblasts to adipocytes [8] has been experimentally demonstrated. Recently we have proposed that caveolae could differently regulate the time course of Shc phosphorylation and Grb2 recruitment by insulin and IGF-I, respectively [9]. While both insulin and IGF-I cause caveolin 1 tyrosine phosphorylation [5,6], the IGF-I effect on phosphocaveolin 1 cellular redistribution is rather different from that of insulin [6]. Finally, a number of IR and IGF-IR substrates, in particular Shc, are enriched in caveolae [10]. The intracellular localization of IRS1 is more complex. It has been proposed that IRS1 is a cytosolic protein, enriched in endosomes [11], which undergoes translocation to plasma membrane upon insulin stimulation [12]. In adipocytes insulin induces IRS1 tyrosine phosphorylation in caveolae [13]. In vitro experiments demonstrated that caveolin 1 is an insulin signalling sensitizer and increases IRS1 tyrosine phosphorylation [14]. However, a direct interaction between IRS1 and caveolin proteins has not been yet demonstrated. Caveolin 1 takes part in IGF-I action in several cell lines [6–8] but the role of caveolin 1 in the IGF-1 mediated IRS1 phosphorylation remains unclear. Aims of this work were to investigate whether IRS1 is tyrosine phosphorylated in caveolae in the presence of IGF-I and whether caveolin 1 directly interacts with IRS1. Here we show that (1) IGF-I induces IRS1 tyrosine phosphorylation in caveolae, (2) caveolin 1 co-immunoprecipitates with IRS1, and (3) IGF-I induces a significant increase of phosphocaveolin 1 and IRS1 co-immunoprecipitation.

Materials and methods

Materials. Anti-IRS1 and anti-caveolin 1 antibodies were obtained from Transduction. ECL reagents were purchased from Santa Cruz Biotechnology. Peroxidase conjugated cholera toxin B subunit, anti-rabbit, and anti-mouse Ig horseradish peroxidase linked were obtained

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from Sigma. Dr. Giorgio Sesti (University Magna Graecia of Catanzaro, Italy) kindly provided cDNA coding for human IRS1.

Cell culture and transfection. R-IGF-IR cells [6] were grown in DMEM supplemented with 2 mM glutamine, 10% FCS, 100 U/ml penicillin G, 100 mg/ml streptomycin sulfate, and G418 500 μg/ml. Subconfluent R-IGF-IR cells were transiently transfected with cDNA coding for human IRS1 complexed with polyethylenimine [15].

Caveolae isolation. Total cell lysates in Na_2CO_3 0.5 M (pH 11) were separated by ultracentrifugation on a discontinuous sucrose gradient and a total of 12 fractions were collected, as described [9].

Immunoprecipitation and immunoblotting. Serum starved cells were stimulated with IGF-I 10 nM for the indicated time in a CO_2 incubator at 37 °C. Then cells were lysed in Na_2CO_3 0.5 M (pH 11). Immunoprecipitations were carried out as described [16]. Immunoprecipitated proteins were separated on 8% SDS–PAGE and transferred to nitrocellulose. Filters, blocked in 5% dried milk, were incubated with primary antibodies for 2 h at room temperature and washed extensively, and secondary horseradish-peroxidase linked antibodies were added for 1 h at room temperature. Bound antibodies were detected using the enhanced chemiluminescence lighting system (ECL), according to manufacturer's instructions. Bands of interest were quantitated by densitometry using the NIH Image software. Ganglioside GM1 was detected as described [6].

Results

R-IGF-IR cells express about 250×10^3 human IGF-IRs that are highly enriched in caveolae [6]. Also IRS1 was detected in caveolae (fractions 5–7) of R-IGF-IR cells, biochemically defined by the co-localization of ganglioside GM1 and caveolin 1 (Fig. 1). As shown in Fig. 2 (left panel), IGF-I caused IRS1 tyrosine phosphorylation in caveolae with a peak after 5 min without any apparent increase of the total amount of IRS1, as detected by anti-IRS1 antibody immunoblot (Fig. 2, right panel). IRS1 contains putative binding sites for caveolin 1 scaffolding domain. To determine whether

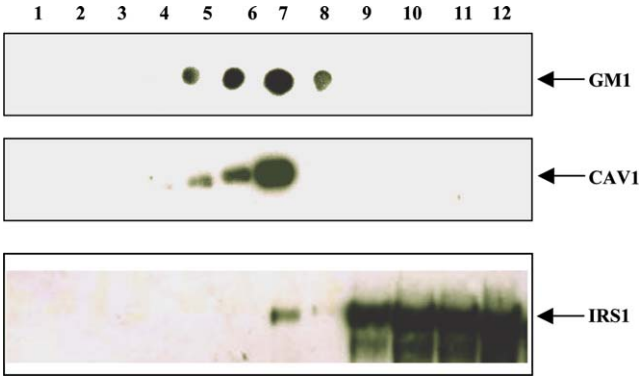

Fig. 1. IRS1 associated with the lipid raft caveolin 1 enriched microdomains in R-IGF-IR cells. Total cell lysate in Na_2CO_3 0.5 M (pH 11) was separated by ultracentrifugation on a discontinuous sucrose gradient and a total of 12 fractions were collected, as described in Materials and methods. The position of the lipid rafts was identified by dot blot of ganglioside GM1 using HRP-conjugated cholera toxin B subunit as a probe (upper panel). Twenty-five microliters from each fraction separated on 12% SDS–PAGE, transferred on nitrocellulose, blotted with an antibody directed against caveolin 1 (CAV 1) or IRS1, and developed by ECL.

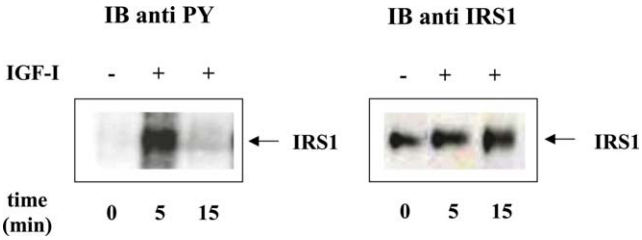

Fig. 2. IGF-I causes IRS1 tyrosine phosphorylation in caveolae of R-IGF-IR cells. Serum starved R-IGF-IR cells were stimulated with IGF-I 10 nM for 5 and 15 min. Twenty-five microliters from caveolae (fractions 5–7) was separated on 8% SDS–PAGE, transferred on nitrocellulose and probed with an anti-phosphotyrosine antibody (left panel) and anti-IRS1 antibody (right panel).

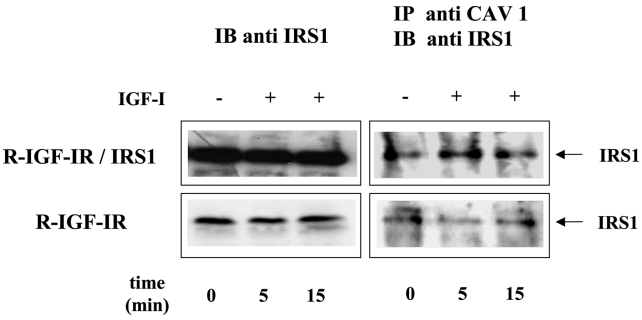

Fig. 3. IRS1 directly interacts with caveolin 1. Serum starved R-IGF-IR and R-IGF-IR/IRS1 cells were stimulated with IGF-I 10 nM for the indicated times and then lysed. (Left panels) Twenty micrograms of proteins from each condition was loaded on SDS–PAGE, transferred on nitrocellulose, and blotted with an antibody directed against IRS1. (Right panels) Cell lysates were immunoprecipitated with an anti-caveolin 1 antibody. Immunoprecipitated proteins were resolved on 8% SDS–PAGE, transferred on nitrocellulose, and blotted with an antibody directed against IRS1.

IRS1 directly interacts with caveolin 1, we utilized R-IGFIR/IRS1 cells, which overexpress both IGF-IRs and IRS1. As shown in Fig. 3, IRS1 coimmunoprecipitated with caveolin 1, both in the absence and in the presence of IGF-I, in a similar amount, suggesting a constitutive interaction between these proteins in caveolae. Superimposable results were obtained in R-IGF-IR cells, which overexpress IGF-IR but not IRS1 (Fig. 3) further supporting a physiological role of this interaction. Since insulin and IGF-I cause phosphorylation of caveolin 1 at the level of tyrosine 14 [4–6], we measured the amount of phosphocaveolin 1 that co-immunoprecipitates with IRS1. Then immunoprecipitation and immunoblot experiments using anti-phosphocaveolin 1 and anti-IRS1 antibodies were carried out in R-IGF-IR/IRS1 cells. As shown in Fig. 4 (upper panel), IGF-I significantly increased (about 6–8-fold) phosphocaveolin 1 and IRS1 co-immunoprecipitation with a peak after 5 min of IGF-I stimulation. This time course is superimposable to that of caveolin 1 phosphorylation induced by IGF-I in total cell lysates (Fig. 4, lower panel) and to the redistribution of phosphocaveolin 1 in the lipid rafts [6].

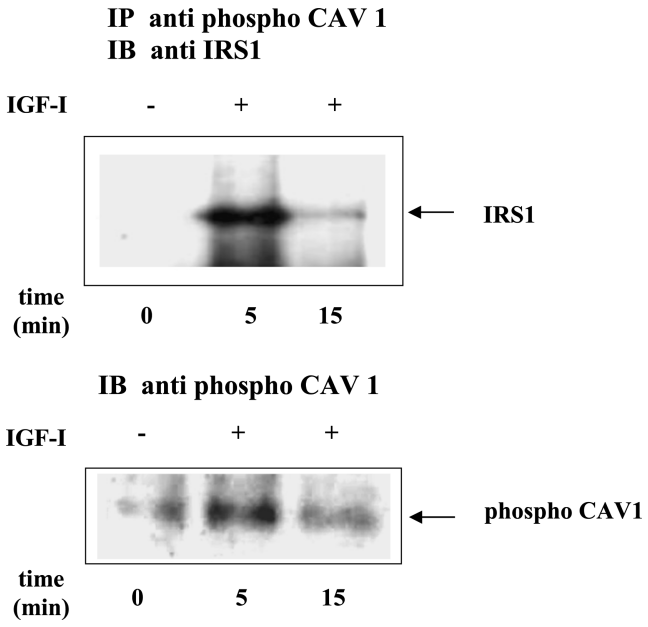

Fig. 4. IGF-I causes phosphocaveolin 1 and IRS1 co-immunoprecipitation. (Upper panel) Serum starved R-IGF-IR/IRS1 cells were stimulated with IGF-I 10 nM at 37 °C for the indicated times and then lysed. R-IGF-IR/IRS1 cell lysates were immunoprecipitated with an anti-phosphotyrosine 14 caveolin 1 antibody. Immunoprecipitated proteins were resolved on 8% SDS–PAGE, transferred on nitrocellulose, blotted with an antibody directed against IRS1, and developed by ECL. (Lower panel) R-IGF-IR/IRS1 cells were stimulated with IGF-I 10 nM for the indicated times. Twenty micrograms from each conditions was loaded on SDS–PAGE 12%, transferred on nitrocellulose, and blotted with an antibody directed against phosphocaveolin 1.

Discussion

The role of IRS1 in the IGF-I signalling is well established [17]. However, the subcellular localization of IRS1 remains controversial. A major amount of IRS1 was detected in the cytoskeletal fraction of adipocytes. This fraction is insoluble, as well as caveolae, in non-ionic detergent [12]. Interestingly, the same cytoskeletal fraction contains also a large amount of p52 Shc that we demonstrated highly enriched in caveolae of R-IGF-IR cells [9]. In CHO cells, IRS1 is localized in the close proximity of plasma membrane [12,18] but IRS1 was also detected in caveolae of adipocytes [13]. Despite these apparently contrasting findings, several data support a role of caveolae in IRS1 activation, at least in the insulin signalling pathway: (a) in HEK293T cells, caveolin 1 and caveolin 3 (but not caveolin 2) increase insulin stimulated IRS1 phosphorylation without affecting IR autophosphorylation [14]; (b) caveolae depletion impairs IRS1 phosphorylation by reducing IR–IRS1 interaction [19]; finally, (c) IRS1 phosphorylation and activation of the downstream substrate Akt were inhibited by β-methyl cyclodextrin treatment which disrupts caveolae [19]. Here we show that IRS1 is present in caveolae of R-IGF-IR cells and it is tyrosine phosphorylated in the presence of IGF-I. In contrast with the previous findings showing that Shc proteins are highly enriched in caveolae [9], the major amount of IRS1 was detected in the cytosolic fractions. These data agree with those observed in adipocytes [13] and CHO cells [12]. Despite the low amount of IRS1 detected in caveolae, experimental findings support a role of this region in the compartmentalization and regulation of IGF-I signalling. First, IRS1 and caveolin 1 co-immunoprecipitate both in the presence and in the absence of IGF-I. Based on the density gradient finding that caveolin 1 was restricted in caveolae, it could be proposed that IRS1 and caveolin 1 co-immunoprecipitate specifically in this compartment. This finding supports a role of caveolin 1 in the targeting of IRS1 to caveolae. Second, caveolin 1 and IRS1 co-immunoprecipitation suggests the existence of a structural link between these two proteins. Caveolin 1 contains a scaffolding domain which recognizes specific caveolin binding motifs localized in signalling proteins such as the insulin receptor [20]. The analysis of human IRS1 sequence reveals the presence of caveolin binding motifs that could explain the co-immunoprecipitation of these proteins. Third, IGF-I increases IRS1 and phosphocaveolin 1 interaction and IRS1 tyrosine phosphorylation in caveolae but does not cause IRS1 recruitment thus suggesting a specific role of IRS1 in these microdomains. Finally, the time course of caveolin 1 phosphorylation in total cell lysates and phosphocaveolin 1 redistribution in the lipid rafts were superimposable with the time course of IGF-I induced IRS1-phosphocaveolin 1 co-immunoprecipitation supporting the existence of a functional link between these IGF-I effects.

Both IRS1 and caveolin 1 act as docking proteins and their binding activity toward SH2 containing proteins is increased by tyrosine phosphorylation [21,22]. The present findings suggest that the IGF-I dependent phosphocaveolin 1–IRS1 physical interaction could further regulate the formation of signalling protein complexes in caveolae.

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