EXPERIMENTAL CELL RESEARCH 318 (2012) 1745–1758

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Insulin receptor substrate 1 expression enhances the sensitivity of 32D cells to chemotherapy-induced cell death

Holly A. Porter[a,c], *Gregory B. Carey*[a,b,d], *Achsah D. Keegan*[a,b,c,d,*]

[a]*Center for Vascular and Inflammatory Diseases, 800 West Baltimore Street, Room 318, Baltimore, MD 21201, USA*
[b]*Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[c]*Molecular Medicine Program, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[d]*Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA*

ARTICLE INFORMATION

Article Chronology:
Received 20 February 2012
Received in revised form
25 April 2012
Accepted 29 April 2012
Available online 28 May 2012

Keywords:
Insulin receptor substrate
Signaling
Annexin A2
Chemotherapy
Cell death

ABSTRACT

The adapters IRS1 and IRS2 link growth factor receptors to downstream signaling pathways that regulate proliferation and survival. Both suppress factor-withdrawal-induced apoptosis and have been implicated in cancer progression. However, recent studies suggest IRS1 and IRS2 mediate differential functions in cancer pathogenesis. IRS1 promoted breast cancer proliferation, while IRS2 promoted metastasis. The role of IRS1 and IRS2 in controlling cell responses to chemotherapy is unknown. To determine the role of IRS1 and IRS2 in the sensitivity of cells to chemotherapy, we treated 32D cells lacking or expressing IRS proteins with various concentrations of chemotherapeutic agents. We found that expression of IRS1, in contrast to IRS2, enhanced the sensitivity of 32D cells to chemotherapy-induced apoptosis. When IRS2 was expressed with IRS1, the cells no longer showed enhanced sensitivity. Expression of IRS1 did not alter the expression of pro- and anti-apoptotic proteins; however, 32D-IRS1 cells expressed higher levels of Annexin A2. In 32D-IRS1 cells, IRS1 and Annexin A2 were both located in cytoplasmic and membrane fractions. We also found that IRS1 coprecipitated with Annexin A2, while IRS2 did not. Decreasing Annexin A2 levels reduced 32D-IRS1 cell sensitivity to chemotherapy. These results suggest IRS1 enhances sensitivity to chemotherapy in part through Annexin A2.

Introduction

Insulin receptor substrate (IRS) proteins 1–6 are large cytoplasmic docking proteins that are important for receptor signaling. IRS1 and IRS2 are widely expressed, while the other family members (IRS3-6) have restricted expression patterns [1]. Most hematopoietic cells express IRS2, but not IRS1 [2,3]. Other cell types, including many cancer cells express IRS1, IRS2, or both [4,5]. IRS3 is restricted to mouse adipose tissue while IRS4 is restricted to human brain, liver, kidney, and thymus. IRS5 and IRS6 have less clear roles [1,6]. IRS1 and IRS2 are encoded by single exons on human chromosome 2q36-37 (mouse chromosome 1), and human chromosome 12q341 (mouse chromosome 8), respectively. These proteins function as large adapters and have many

*Corresponding author at: Center for Vascular and Inflammatory Diseases, 800 West Baltimore Street, Room 318, Baltimore, MD 21201, USA. Fax: +1 410 706 8234.
E-mail addresses: hport001@umaryland.edu (H.A. Porter), gcarey@som.umaryland.edu (G.B. Carey), akeegan@som.umaryland.edu (A.D. Keegan).

0014-4827/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexcr.2012.04.020

protein binding motifs that allow them to interact with other proteins. IRS1 and IRS2 have a well conserved pleckstrin homology (PH) domain and a phosphotyrosine binding (PTB) domain, which interact with phospholipids and phosphorylated receptors, respectively. Furthermore, they both have a large COOH-terminal region containing many potential sites for tyrosine and serine/threonine phosphorylation [7–9].

IRS1 and IRS2 have been shown to mediate signaling from activated cell surface receptors including the receptors for insulin, insulin-like growth factor 1 (IGF-1), prolactin, growth hormone (GH), vascular endothelial growth factor (VEGF), and several cytokine receptors including those for interleukin (IL)-4, -9, -13, and -15 [1,4,7,10]. When these receptors are activated by ligand binding, the IRS proteins are recruited to the receptors by interaction of their PTB domains with phosphorylated residues within the receptor tails. IRS1 and IRS2 are then phosphorylated on multiple residues by receptor-associated kinases [7,11–15]. IRS1 and IRS2 have ~20 potential tyrosine phosphorylation sites in their COOH-terminal region. Once phosphorylated, IRS1 and IRS2 can recruit additional signaling molecules and activate downstream signaling pathways. Phosphorylated IRS1 and IRS2 have been shown to interact with SHP2, Fyn, Grb-2, Nck, Crk, and p85, the regulatory subunit of phosphoinositide-3-kinase (PI-3-K) [6,14,16–19]. IRS1 and IRS2 have four and two possible p85 binding sites, respectively. The p85 subunit contains an N-terminal SH3 domain and C-terminal SH2 domain that mediate its interaction with the p110 catalytic subunit. The p110 subunit can phosphorylate membrane lipids, which are involved in the activation of several kinases, including AKT (also known as protein kinase B), that play an important role in cell survival. The p110 subunit can also phosphorylate, by a less well understood mechanism, Ser/Thr protein residues, including those of IRS1 [14,15].

While the tyrosine phosphorylation of certain residues leads to enhanced recruitment of downstream pathways, phosphorylation of IRS1 has also been shown to reduce certain protein interactions. IRS1 has been shown to interact with the DNA repair protein RAD51 via its PTB domain; tyrosine phosphorylation induced by IGF-1 suppressed this interaction [9]. Furthermore, IRS1 Ser/Thr phosphorylation by multiple kinases, including JNK, mTOR, and PKC, has been shown to interrupt the interaction of IRS1 with activated receptors and may promote its interaction with 14–3–3 proteins. Phosphorylated serines may also sterically prevent other proteins from binding to IRS1. Activation of Ser/Thr kinase activity may create a negative feedback loop to regulate IRS1 function [4,15,20–24]. IRS proteins are also regulated by ubiquitination, which can be mediated by Ser/Thr phosphorylation, leading to proteasomal degradation [1,23–25]. On the other hand, in some cases serine phosphorylation of IRS1 may actually promote cell signaling. For example, in human prostate cancer cells the serine phosphorylation of IRS1 inhibited its tyrosine phosphorylation and promoted cell adhesion while decreasing cell motility because of enhanced interactions with $\alpha5\beta1$ integrins [26].

Chemotherapy involves the use of anti-cancer drugs to treat advanced or metastatic cancers systemically. Chemotherapeutic agents can induce double-strand breaks in DNA causing proliferating cells to growth arrest and then undergo apoptosis [9,27–32]. Many patients show inherent or acquired resistance to chemotherapy and in some cases this is due to alterations in the levels of pro-apoptotic and anti-apoptotic proteins [28,29,31]. Despite similar structure and function, IRS1 and IRS2 have also been implicated in cancer progression by differentially regulating cell survival, growth, proliferation, and motility [4,6,7,15,33–36]. It has been postulated that differences in functions of IRS1 and IRS2 in cancer progression may be due to difference in tissue distribution, subcellular localization, or protein recruitment [19,34]. However, the roles of IRS1 and IRS2 in regulating responses to chemotherapy are unclear. Therefore, to determine the role of IRS1 and IRS2 in the protection of cells from chemotherapy-induced cell death, we utilized the 32D cell model system. The 32D cell line is an IL-3 dependent myeloid progenitor line that lacks expression of IRS1 and IRS2. These cells have been used extensively as models for growth factor receptor signaling, proliferation, and regulation of apoptosis [3,34,37–40]. We found that IRS1, but not IRS2, expression sensitized 32D cells to chemotherapy-induced caspase-mediated cell death. Although this was not due to changes in expression of pro- or anti-apoptotic proteins, we did find that cells expressing IRS1 expressed higher levels of Annexin A2. In 32D-IRS1 cells IRS1 and Annexin A2 were both located in cytoplasmic and membrane fractions. We also found that IRS1 coprecipitated with Annexin A2, while IRS2 did not. Decreasing Annexin A2 levels reduced 32D-IRS1 cell sensitivity to chemotherapy. These results showed that enhanced expression of IRS1 led to enhanced responses to chemotherapy and suggest that Annexin A2 may participate in this response.

Materials and methods

Materials

32D cells were obtained from Wang et al. [37]. Recombinant IL-4 (rIL-4) was purchased from R &D Systems and used at a final concentration of 10 ng/ml. The caspase inhibitor, Q-VD-OPh, was also purchased from R & D Systems and used at a final concentration of 10 μM. Neomycin (G418) and tetramethylrhodamine (TMR) were purchased from Invitrogen. Vincristine, etoposide, taxol, and daunorubicin were purchased from Sigma-Aldrich. Taxol was also purchased from LC Laboratories. Propidium Iodide (PI) was purchased from Sigma-Aldrich. Antibodies for BCL-xL, pBAD(Ser136), BAD, BAK, GAPDH, Sp1, and Annexin II were purchased from Santa Cruz Biotechnology. Antibodies for pAKT(4 7 3), AKT, BAX, and BCL-2(50E3) were purchased from Cell Signaling Technologies. Antibody for alpha-tubulin was purchased from eBioscience. Antibody for HSP90 was purchased from Stressgen. Antibodies for IRS1 and IRS2 were purchased from Upstate Biotechnology. Imperial protein stain was purchased from Thermo Scientific.

Cell culture and reagents

32D cells transfected with either IRS1 or IRS2 were maintained in complete RPMI 1640 (Lonza) supplemented with 10% fetal bovine serum (FBS), 1% glutamine, 1% pen-strep, plus 5% WEHI-3B supernatant as a source of IL-3 [3,37]. 32D cells transfected with both IRS1 and IRS2 were maintained in complete RPMI plus 5% WEHI-3B supernatant and 800 μg/ml neomycin (Invitrogen). WEHI-3B supernatant was prepared by growing WEHI-3B cells to terminal