Insulin Receptor Substrate Regulation of Phosphoinositide 3-Kinase

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Abstract

Insulin receptor substrates (IRS) serve as downstream messengers from activated cell surface receptors to numerous signaling pathway cascades. One of these pathways, phosphoinositide 3-kinase (PI3K), frequently displays aberrant function in the setting of cancer. IRS proteins are capable of both regulating and activating PI3K, depending on the cell of origin. As such, both prohost and protumor functions have been described for IRS proteins in human cancers. IRS proteins may eventually serve as biomarkers of PI3K activity, and serve a much-needed role as a guide to using targeted pathway therapy. Additionally, IRS-1 could be indirectly targeted in lung cancer, by inhibiting neutrophil elastase, which functions to degrade IRS-1 in lung tumor cells, thereby generating PI3K hyperactivity. Clin Cancer Res; 17(2); 206–11. ©2010 AACR.

Background

Insulin receptor substrates (IRS) are signaling adaptor proteins that function as intermediates of activated cell surface receptors, most notably for the insulin receptor (IR) and insulin-like growth factor receptor (IGF-IR; refs. 1–3). More recently, IRS proteins have been shown to signal downstream of integrin, cytokine, and steroid hormones receptors as well (4, 5), although these functions are poorly understood when compared with the “canonical” (IR- and IGF-IR-mediated) properties of IRS proteins. By mediating the activities of these receptors, IRS proteins interface with several signaling pathways, thereby impacting numerous aspects of cell behavior, including metabolism, motility, survival, and proliferation (Fig. 1). The majority of IRS protein research to date has centered upon the study of glucose metabolism and the pathogenesis of diabetes. Reports pertaining to the roles of IRS proteins in cancer progression are beginning to emerge (6).

Six IRS proteins have been described; however, IRS-3 is expressed only in rodents (7), IRS-4 displays limited tissue expression (brain and thymus; ref. 8), and IRS-5 and IRS-6 are structurally dissimilar from the others (9). Therefore, most of the attention has been focused on IRS-1 and IRS-2, both of which are widely expressed. IRS proteins share similar structural domains including an N-terminal pleckstrin homology domain and a phospho-tyrosine binding (PTB) domain, which is required for binding NPEY motifs in the juxtamembrane region of ligand-activated IR and IGF-IR (10). The carboxy terminus contains numerous serine and tyrosine phosphorylation sites that bind PTB containing src-homology-2 (SH2) proteins, including p85, Grb2, Nck, the phosphotyrosine phosphatase SHP2, Fyn, and others (11). Although IRS proteins are not catalytically active, they are capable of impacting numerous signaling cascades via interaction with SH2 proteins.

Despite sharing binding partners and structural similarities, IRS-1 and IRS-2 functions are not entirely overlapping. IRS-1−/− mice display low birth weight and glucose intolerance, but do not develop overt diabetes (12). The generation of these mice led to the discovery of IRS-2, which was believed to compensate for the loss of IRS-1 and prevent additional metabolic derangements. IRS-2−/− mice have subsequently been shown to develop diabetes as a consequence of decreased β-cell function and insulin resistance (13). Therefore, IRS-1 and IRS-2 possess both overlapping and unique properties, although their relative contribution to cancer growth and invasiveness has yet to be elucidated.

Though IRS proteins signal through many pathways, their predominant function seems to be activation and/or regulation of the phosphoinositide 3-kinase (PI3K) and extracellular signal regulated kinase (ERK) pathways. PI3K is a heterodimer with separate regulatory (p85) and catalytic subunits (p110). In its resting state, PI3K exists as an inactive p85-p110 complex. Upon the activation of a receptor tyrosine kinase (RTK), meaning phosphorylation of its cytoplasmic tail, the p85-p110 complex is recruited to the receptor by interaction of an SH2 domain on p85 with phosphotyrosine residues on the RTK (14). This interaction is believed to release the inhibitory effects of p85 on the catalytic p110 (15). p110 is now able to interact with its lipid substrates, the phosphatidylinositol, and convert PIP2 to PIP3. Recruitment of PI3K by RTK also puts p110 in close proximity to these lipid substrates residing in the plasma membrane. The major exception to this schema is that PI3K can be activated by signal adapter proteins, such as IRS-1 and IRS-2, rather than by RTKs.
themselves (5). Of note, IRS-mediated activation of PI3K requires that phosphorylated YMXYM motifs occupy both SH2 domains within p85 (16).

Generation of PIP3 by activated PI3K near the plasma membrane results in interaction with, and subsequent phosphorylation of, its primary substrate, Akt (17). Once activated, pAkt uses extensive downstream signaling pathways to enhance tumor viability in one of three ways: cell survival, cell proliferation (number), and cell growth (size; ref. 18). The avoidance of apoptosis, achieved by the direct phosphorylation of BAD by pAkt, is generally considered the predominant function of PI3K/Akt in cancer cells (19). However, PI3K/Akt also promotes tumor cell proliferation by causing an accumulation of cyclin D1, which regulates G1/S phase transition (20). This is accomplished by inhibition of p27, p21, and glycogen synthase kinase-3β (GSK3β), which target cyclin D1 for proteosomal degradation, when active (21, 22).

PI3K activity seems to be regulated in two ways. The first is simply the activation of the p85 subunit, which maintains p110 in an inactive state at baseline. The second is the constitutively active negative repressor, phosphatase and tensin homolog (PTEN). PTEN regulates the output of PI3K by dephosphorylating PIP3 back to PIP2 (23). Mutation in PTEN is relatively common in cancers, and has been linked to PI3K hyperactivity in several, including prostate carcinoma, hepatocellular carcinoma (HCC), melanoma, renal-cell carcinoma, and glioblastoma, among others (24–28). Interestingly, PTEN mutation is rare in some cancers, including lung cancer (29). The common explanations for PI3K hyperactivity in the setting of preserved PTEN expression has been activation of PI3K by K-ras and genetic mutations within the PI3K pathway (e.g., PIK3CA), both of which possess the ability to by-pass regulatory machinery (30–32).

Although generally considered positive effectors of growth factor, IRS proteins may, in fact, function as homeostatic regulators of PI3K output in certain tissues. Supporting evidence of this theory includes the fact that IRS-p85 interaction takes place within the cytosol, pulling PI3K away from its lipid substrates and creating compartmentalization phenomena for signaling (33). Furthermore, IRS proteins display low potency for ligand interaction. As an example, IRS-1-Grb2 binding results in nearly 10-fold less pathway output when compared with other common Grb2-binding partners (34).

Both IRS-1 and IRS-2 are commonly overexpressed in HCC, which is characterized by IR and IGF-IR signaling hyperactivity (39, 40). IRS-1 overexpression in HCC cell lines prevents TGF-β-induced apoptosis (41). In fact, transfection of these cells with a dominant-negative IRS-1 reverses their malignant phenotype (42).

The role of IRS-1 in breast cancer has been difficult to elucidate, as both prohost and protumor functions have been described. Simple overexpression of IRS-1 in MCF-7 breast cancer cells accelerates their growth, whereas IRS-1 gene silencing ultimately results in apoptosis, at least under serum-free conditions (43, 44). IRS-1 and IRS-2 transgenic mice both display enhanced tumor growth, metastasis, and resistance from apoptosis (45). These mice develop mammary gland hyperplasia early in life, and display unusual tumor histology, and not the typically encountered adenocarcinoma (46). Thus, these overexpression studies may not be representative of pathophysiologic properties of IRS proteins in human cancers. As such, IRS-1–silenced tumor xenografts actually displayed increased metastasis (47), consistent with a prohost role for IRS-1. Additionally, studies of IRS-1 in human breast cancer show that IRS-1 expression is lost in clinically advanced cases (48).

We have recently described a prohost role for IRS-1 in lung cancer (49). While investigating the role of neutrophil elastase in lung cancer, we observed that neutrophil elastase–deficient tumors in the Lox-Stop-Lox-K-ras (LSL-K-ras) model of lung adenocarcinoma (45) accumulated intracellular IRS-1 protein, whereas neutrophil elastase–sufficient tumors contained scant IRS-1. We were able to show that IRS-1 is an intracellular proteolytic target for neutrophil elastase, which induced cellular proliferation and pAkt production upon the degradation of IRS-1. Ultimately, we discovered that the loss of IRS-1 functioned to increase the pool of bioavailable PI3K, rendering p85 free to interact with the more potent growth factors present in lung cancer cells, especially the platelet-derived growth factor (PDGF) and receptor (PDGFR) complex (51). Consistent with this concept, IRS-1 gene silencing in lung cancer cells resulted in cellular proliferation and pAkt production, whereas IRS-1 overexpression induced cell cycle arrest. Furthermore, a correlation of the presence of neutrophil elastase with the absence of IRS-1 was established in human lung adenocarcinomas. Thus, IRS-1 is capable of both growth promoting and growth regulatory functions in cancers, depending on the cell of origin.

### Clinical-Translational Advances

With respect to IRS proteins, studies translating the findings observed in murine models to human cancers are sparse. However, the existing studies do support the hypothesis that IRS-1 can function both for and against the host, depending on the cell of origin. Nearly all studies suggesting that IRS1 protein function as positive effector of growth factor were done in metabolically active tissues, such as myocytes, adipocytes, and hepatocytes (3). As such, IRS-1 seems to promote tumor growth in malignancies that
arise from such cell types including leiomyosarcomas, myosarcomas, liposarcomas, rhabdomyosarcomas, and HCCs (52). Interestingly, the single study of IRS-1 in non–small cell lung cancer (NSCLC) showed that the loss of IRS-1 expression correlated with increased tumor growth (53), consistent with our findings with respect to IRS-1 in murine models of lung adenocarcinoma. Further evidence that IRS-1 homeostatically regulates PI3K in cancer can be elucidated from the study of G972R polymorphism. The presence of the G972R polymorphism within IRS-1 decreases interaction with p85 (54), such that PI3K will be activated by other growth factors within the cell. This polymorphism has been associated with an increased risk of prostate cancer (55).

Figure 1. IRS-1 regulates downstream signaling of IR/IGF-IR. IRS-1 is a homeostatic regulator of PI3K signaling in lung tumor cells. IRS-1 has serine and tyrosine phosphorylation sites that bind SH2 domain–containing proteins including p85, Grb2, and SHIP2, among others. IRS-1 recruits PI3K and MEK/ERK via interaction with the regulatory p85 subunit and GRB2, respectively. The catalytic subunit of PI3K, p110, is now available to convert PIP2 to PIP3. PIP3 activates PDK1, which subsequently phosphorylates AKT, enhancing cell survival, proliferation, and growth. Downstream effectors of AKT inhibit apoptosis via inhibition of Bad, Bim, Bax, and caspase 9, and activation of BCL-XL and Bcl-2. Tumor cell proliferation is promoted by the inhibition of GSK3, which targets cyclin D1 for proteasomal degradation. Increased protein synthesis results from activation of the mTOR pathway. The MEK/ERK pathway also promotes proliferation via interaction of IR/IGF-IR with IRS or Shc proteins. A feedback loop exists between the PI3K/AKT signaling pathway and IRS-1, in which IRS-1 is degraded through the ubiquitin-proteasome degradation pathway. The above pathways have been well established in metabolically active tissues including adipose and muscle. The above figure, however, describes an alternative to those established paradigms. In lung cancer cells, the PI3K/AKT pathway is weakly activated by IRS-1. IRS-1 acts homeostatically to prevent activation of PI3K by more potent mitogens, including PDGF. Therefore, the loss of IRS-1 increases the amount of available PI3K, which can then be activated by these mitogens causing much greater pathway activation. As shown, neutrophil elastase (NE) can enter tumor cells and degrade IRS-1 during tumor-associated neutrophilic inflammation allowing other RTKs to control PI3K signaling.

Inhibition of PI3K, of which IRS-1 is an integral component, is currently under investigation in numerous early phase trials (56, 57). It is generally accepted that the successful application of PI3K inhibitors (and all pathway-directed therapies for that matter) will require biomarkers predictive of tumor response to a given therapy. Early studies employing targeted pathway inhibition in human cancers have already highlighted this point. Specifically, the presence of K-ras mutation in colorectal cancer has been used to predict response to anti–epidermal growth factor receptor (EGFR) therapy (58), and Her2 overexpression has been used to predict response to trastuzumab (herceptin; ref. 59).

No biomarkers have been validated for response to PI3K inhibition. Some markers are predictive of prognosis, such as combined analysis of PTEN and PIK3CA status, and may yet prove useful in predicting response to PI3K antagonism (60–62). The first step toward using IRS-1 and/or IRS-2 in this capacity will be to acquire the necessary translational studies to clearly define the impact of the presence or absence of IRS-1/IRS-2 on PI3K activity level,
and to correlate these findings with meaningful clinical outcomes data for each distinct cancer subtype. As described above, it is likely that IRS-1-P13K interaction will produce differential effects on cell behavior depending on the cell of origin. Therefore, it is plausible that IRS-1 levels will predict opposing outcomes when comparing HCC to NSCLC, for example. We propose that the loss of IRS-1 in lung cancers will result in paradoxical hyperactivity of P13K, independent of genetic mutations that commonly cause P13K hyperactivity (K-ras, PIK3CA, PTEN, etc.). P13K hyperactive tumors (pAkt positive) that are devoid of IRS-1 may represent a subclass amenable to inhibition of P13K, and possibly to MEK/ERK antagonism. Commonly employed expression profiles will continually fail to identify changes in IRS-1 expression, as IRS-1 is posttranslationally degraded by components within the tumor microenvironment (neutrophil elastase). Therefore, detailed analyses of IRS-1 protein content (immunohistochemistry, immunoblot) and gene expression (quantitative PCR) must be entertained to identify the nature of IRS-1 loss in each cancer type, and its impact on P13K activity and clinical outcomes.

The most logical way to manipulate tumor IRS-1 content for therapeutic benefit would be to use an indirect approach. IRS-1 loss in human lung adenocarcinoma seems to be a result of neutrophil elastase–mediated degradation. Therefore, antagonism of neutrophil elastase within the tumor microenvironment should preserve IRS-1 content within tumor cells and maintain homeostatic regulation of P13K, at least in lung tumors. We employed this approach to successfully reduce lung tumor burden in LSL-K-ras tumor–bearing mice using the neutrophil elastase inhibitor ONO-5046. Neutrophil elastase inhibitors were previously employed expression profiles will carry out clinical trials in COPD (large cohort size, lack of adequate phenotypic markers to determine response, etc.) and the likelihood that other elastin-degrading enzymes (i.e., matrix metalloproteinases) will drive disease progression independent of neutrophil elastase (63–65). Despite these difficulties, some neutrophil elastase inhibitors are currently undergoing early phase investigation in human COPD (66), and if they prove safe, could readily be tested in human lung adenocarcinoma. Plans to do such studies are underway within our group.

Conclusions

IRS proteins interface with essential signaling pathways commonly implicated in tumor development and progression. Current data suggest that IRS proteins may function either for or against the host in the setting of cancer, depending upon the cell of origin. Further translational studies will be required to determine the impact of IRS-1 and IRS-2 expression or loss on outcomes in various human malignancies. These data may prove useful for the development of IRS protein levels as biomarkers of response to emerging targeted pathway-inhibiting therapies. Preservation of IRS-1 levels within lung tumor cells by inhibiting the IRS-1 degrading enzyme neutrophil elastase may prove an effective therapeutic strategy for patients with NSCLC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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