Published in final edited form as:

J Mammary Gland Biol Neoplasia. 2008 December ; 13(4): 415. doi:10.1007/s10911-008-9101-9.

Insulin Receptor Substrates (IRSs) and breast tumorigenesis

Bonita Tak-Yee Chan and Adrian V. Lee

Lester and Sue Smith Breast Center, Departments of Medicine and Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

Abstract

Insulin receptor substrate (IRS)-1 and IRS-2 are adaptor proteins in the insulin-like growth factor I (IGF-I)/IGF-I receptor (IGF-IR) pathway that mediate cell proliferation, migration, and survival. In addition to their role as scaffolding proteins in the cytoplasm, they are able to translocate into the nucleus and regulate gene transcription. IRS levels are developmentally and hormonally regulated in the normal mammary gland and both are essential for normal mammary gland bud formation and lactation. Both IRS-1 and IRS-2 are transforming oncogenes, and induce transformation and metastasis *in vitro* and *in vivo*. In breast cancer IRSs have unique functions, with IRS-1 being mainly involved in cell proliferation and survival, whereas IRS-2 has clear roles in cell migration and metastasis. In this review we will discuss the roles of IRSs in mammary gland development and breast cancer.

Keywords

Insulin receptor substrate; IRS-1; IRS-2; IRS1; IRS2; mammary gland; tumorigenesis; breast cancer; transformation; transgenic mice

1) The IRS family of proteins

There are six members of the IRS family of signaling adaptor proteins (IRS-1-6) (1–6). IRS-1 and IRS-2 are widely expressed in tissues, including brain, muscle, heart, adipocyte, kidney, ovary and mammary gland (7–10), whereas IRS 4 is limited to the brain and thymus (5). While IRS-3 is expressed in rodents, a paralog has not been identified in humans (4). IRS-5 and IRS-6 are considered ‘distant relatives’ from the rest of the family and are more similar to each other than to the other IRS proteins (6).

Owing to their wide tissue expression in rodents and humans, IRS-1 and IRS-2 are the most well-studied. IRS-1 and IRS-2 are highly similar to each other in protein structure (2,3,11). Both contain a highly conserved N-terminus, with a plextrin homology (PH) and phosphotyrosine binding (PTB) domains which mediate the binding to insulin receptor (IR) and insulin-like growth factor receptor (IGF-IR) (11–15). In their C-termini, IRSs contain numerous tyrosine and serine residues that serve as docking sites for Src-homology 2 (SH2) containing proteins (11,16–18). Upon phosphorylation of the tyrosine and serine residues, downstream adaptor proteins are recruited and activated (11,19). The best characterized downstream effectors are PI3K and ERK1/2 (20–24). Ligand-activated IGF-IR and IR signaling pathways that involve direct IRS binding are known as canonical IRS signaling pathways (1,3,11,24,25). However, IRS-1 and IRS-2 are involved in other non-canonical signaling pathways independent of binding to IGF-IR/IR, including cytokine (e.g. interleukins

*Corresponding author: Adrian V. Lee Ph.D., Breast Center, One Baylor Plaza, MS:600, Room N1110, Baylor College of Medicine, Houston, TX 77030. Tel: 713-798-1624. Fax: 713-798-1642. avlee@breastcenter.tmc.edu.

such as IL-4) or hormone (e.g. prolactin and growth hormone)-induced signaling pathways (24,26–28). For example, cytokine receptors can activate JAK cytoplasmic tyrosine kinases to phosphorylate and activate the IRSs (29).

IRS-1 and IRS-2 also exhibit non-canonical functions inside the nucleus (for review see (24)). A number of studies have shown nuclear translocation of IRS proteins (30,31). Interestingly, IRS proteins don't have a nuclear translocation signal, but their translocation is dependent on their PTB domains (32). Translocation of IRS proteins is promoted by stimulation with IGF-I (31) or certain oncogenes (32). Inside the nucleus, IRS-1 and IRS-2 regulate transcription of genes involved in different stages of cancer progression. Nuclear IRS-1 associates with estrogen receptor α (ER α) to form a complex (30). The IRS-1/ER α complex binds to the estrogen-responsive element (ERE) in the pS2 gene promoter and regulates ER α transcription (30,31). Nuclear IRS-1 interacts with β -catenin, which is induced in the process of epithelial-mesenchymal transition, and IRS-1 also regulates the transcription of cyclin D1 (33). In fact, it has been shown that nuclear IRS-1 binds to cyclin D1, c-myc and rDNA promoters to increase their promoter activity (34). Furthermore, nuclear IRS-1 attenuates DNA repair by interaction with RAD-51 in polyomavirus JC T-antigen-induced double strand break of DNA (35,36). Similar to IRS-1, IRS-2 also translocates into the nucleus. Nuclear IRS-1 and/or IRS-2 has been shown to regulate RNA polymerase I activity by binding to upstream binding factor 1 and thus altering ribosomal RNA synthesis (37,38). Taken together, these studies suggest an important role of IRS proteins in regulating the transcriptional level of a number of genes.

2) IRSs in mammary gland development and function

2.1) IRS levels during postnatal development

A number of studies have shown the significance of the IGF system in mammary gland development (for review see other articles in this journal). We showed that IRS-1 and IRS-2 proteins are expressed in all stages of mammary gland development (39). In nulliparous mice, both proteins are expressed in epithelial ducts and terminal end buds (TEBs), and only rarely myoepithelium or stroma. Interestingly, we found that IRS-1 expression showed a unique punctuate pattern in the ducts. Importantly, the same pattern has been shown for expression of steroid hormone receptors, including ER and progesterone receptors, suggesting that IRS-1 expression is regulated by steroid hormones (40–43). Injection of IGF-I into virgin mice caused a rapid increase in the phosphorylation of IRS-1 supporting the notion that IRS-1 may mediate IGF-I-induced development (44). In contrast to IRS-1, IRS-2 is expressed in a more uniform pattern and shows a more intense expression in the TEBs (39). Interestingly, IRS-2 is expressed in ER negative cap cells while IRS-1 is absent. During pregnancy, the levels of both proteins increase by about 2-fold and continue increasing until a peak at lactation. At lactation, IRS-1 and IRS-2 proteins are 20 and 10-fold higher than in the virgin gland. A rapid and dramatic loss of both protein levels in the mammary gland immediately follows lactation. By the time of involution, both proteins are completely lost.

2.2) Effects of IRS gene deletion on mammary gland development

2.2.1) Postnatal development—To understand the roles of IRS-1 and IRS-2 in mammary gland development, we examined IRS-1 and IRS-2 null mice. To our surprise we found that FVB/IRS-1-null (39) and FVB/IRS-2 null (unpublished data) mice both showed normal mammary gland development. Both lines of mice underwent normal pregnancy-mediated development of the gland, and both were able to lactate (albeit with slightly reduced efficiency as discussed later).

2.2.2) Mammary bud formation—The lack of phenotype in both IRS-1^{-/-} and IRS-2^{-/-} mouse mammary glands suggested a compensatory role in mammary gland development under

IRS deficiency. Indeed Hadsell *et al.* found elevated IRS-2 tyrosine phosphorylation in the mammary gland of IRS-1^{-/-} mice (45). Therefore, IRS-1/2 double knockout mice would be a better model for testing their roles in mammary gland development, however, IRS-1/2 double knockout mice are embryonic lethal. To circumvent this problem, development of E14.5 embryonic mammary bud transplants were studied in IRS-1^{-/-}/IRS-2^{-/-} mice (46). Staining with the epithelium marker p63 and mesenchymal differentiation marker androgen receptor showed reduced-sized mammary buds with defective epithelial and mesenchymal differentiation in IRS-1/2 double deficient mouse embryos. Mesenchymal proliferation and condensation were decreased in the mammary buds of these mouse embryos. Importantly, epithelial proliferation was not affected suggesting that a defect in mesenchyme-leading migration of epithelial progenitor cells, rather than reduction in proliferation, resulted in smaller mammary buds.

2.2.3) Lactation—Owing to the significant increase of IRS-1 and IRS-2 proteins in the mammary gland during the immediate postpartum period, the role of both proteins during lactation in IRS-1^{-/-} and IRS-2^{-/-} mice was investigated. Hadsell *et al.* showed that lactational capacity was reduced in both IRS-1^{-/-} and IRS-2^{-/-} mice which resulted in pups with lower body weight (45). Analysis of the milk composition in IRS-1^{-/-} mice revealed a decline in lactose content, however, the reduction was not related to the milk protein gene expression or mammary secretory cell carbohydrate metabolism.

3) IRSs as transforming oncogenes in breast cancer

Surmacz's group was the first to show the transforming potential of IRS-1 in mouse embryo fibroblasts (MEFs) (47), and subsequently it was found that IRS-1 actually bound SV40-T antigen and participated in its transforming ability. Overexpression of IRS-1 in NIH3T3 fibroblasts increased phosphorylation of IRS-1 and activation of ERK 1/2 and resulted in transformed foci and colony formation in soft agar (48). Supporting this, IRS-1 co-operates with Ras in the transformation of 32D cells (49,50).

3.1) IRS-mediated transformation of mammary epithelial cells

Previous studies showed that immortalized, non-transformed human mammary epithelial MCF-10A cells form polarized acini in three dimensional (3D) culture (51). Overexpression of either IRS-1 or IRS-2 induced extensive proliferation and disrupted the polarity in MCF-10A cells grown in 3D culture, with the formation of disrupted acini with filled lumen (52). Although the mechanism is still unknown, similar results from ErbB2-overexpressed MCF-10A cells in 3D culture have been reported (53). ErbB2 disrupted the polarity complex by binding to Par 6. Whether IRS-1 and IRS-2 undergoing a similar mechanism remains to be determined.

3.2) Mammary tumorigenesis in IRS transgenic mice

In order to understand the oncogenic capability *in vivo*, we generated transgenic mice with overexpression of either IRS-1 or IRS-2 in the mammary gland (52). MMTV-HA-IRS-1 or MMTV-HA-IRS-2 mice both developed mammary gland hyperplasia, tumors, and metastasis. In both mice, preneoplastic ductal hyperplasia appeared as early as 24 weeks of age, and multiple mammary gland lesions by around 52 weeks of age. Analysis on the histopathology of these tumors showed highly differentiated tumors with only 40% and 20% being mammary adenocarcinomas in MMTV-HA-IRS-1 and MMTV-HA-IRS-2 transgenic mice, respectively. Moreover, metastatic lung tumors were found in 33% and 40% of IRS-1 and IRS-2 mice, respectively. Although the mechanism of the IRS-induced tumorigenesis in these transgenic mice remains unclear, both proteins were found to associate with β -catenin resulting in highly differentiated cell lineages, detectable putative progenitor cells and extensive squamous differentiation in the tumors.

3.3) Modulation of mammary tumor metastasis in IRS-knockout mice

The effects of IRSs on mammary cancer metastasis have been studied by Leslie Shaw's group (54–56). Using IRS-1^{-/-} and IRS-2^{-/-} mice crossed with the oncomouse model MMTV-PyV-MT, they found a significant increase in lung metastasis in MMTV-PyV-MT/IRS-1^{-/-} mice which was associated with activated Akt/mTor signaling. Interestingly, they found increased IRS-2 levels and tyrosine phosphorylation in IRS-1^{-/-} tumor cells (similar to the elevation found in the normal mammary gland of IRS-1^{-/-} mice) suggesting that IRS-2 compensates for the loss of IRS-1 in tumor metastasis. In contrast, metastasis is highly suppressed in MMTV-PyV-MT/IRS-2^{-/-} mice. Analysis of the mammary tumor cells from these mice showed significantly reduced invasion in Matrigel and an increase in apoptosis.

Transgenic and knockout studies have shown divergent roles for IRSs in cancer. IRS-1 and IRS-2 are redundant for primary tumor growth, as both proteins when expressed individually can cause mammary transformation, and reduction of IRS-1 or IRS-2 doesn't affect PyV-MT-induced tumor growth, suggesting that either alone is sufficient for both transformation and maintenance of tumor growth. In stark contrast, this redundancy is not observed in metastasis, where loss of IRS-2 clearly impedes the process. One potential explanation for this observation is that IRS-1 and IRS-2 may share the capacity to generate signals for transformation and primary tumor growth, whereas only IRS-2 is able to promote signals for metastasis. For example, studies in breast cancer cell lines described below have shown that IRS-2 is a major regulator of motility and migration whereas IRS-1 is mainly important for proliferation. Alternatively IRS-2 may selectively activate survival signals required for metastasis. This notion of specific signals downstream of IRSs and a lack of redundancy has previously been shown in brown adipocytes.

4) IRSs in human breast cancer

4.1) IRS expression in breast cancer cells lines, and modulation of IGF-regulated growth and migration

A number of studies have shown roles for IRS-1 and IRS-2 in proliferation and migration downstream of IGF-IR in breast cancer cells. Suppression of IRS-1 blocked more than 70% of cell growth and led to significant apoptosis in MCF-7 breast tumor cells (57) while overexpression of IRS-1 allowed growth under serum-free conditions (58). Furthermore, IRS-1 increased the aggressiveness of BT-20 cells, allowing them to grow in soft-agar and form larger tumors *in vivo* (59). Interestingly, nuclear IRS-1 was found to associate with cell cycle gene promoters including c-myc and cyclin D1 in BT-20 breast cancer cells stimulated with IGF-I (59). Conversely, a recent study showed that IRS-1 formed a complex with androgen receptor (AR) and translocated into the nucleus where it upregulated the transcriptional activity of AR (60).

While IRS-1 prevails in cell proliferation, IRS-2 is more preferentially involved in cell motility. The different roles of IRS-1 and IRS-2 in mammary tumorigenesis were shown in T47D-YA cells which did not express both IRS-1 and IRS-2 (61). Transfection of IRS-1 and IRS-2 led to IGF-I-stimulated cell proliferation and motility, respectively. Both IRS-1 and IRS-2 activated PI3K, however, only IRS-1 was found to phosphorylate ERK 1/2 that promoted cell growth. The downstream signals in IRS-2-mediated cell motility remained to be determined. A role for IRS-2 in cell migration rather than proliferation has also been shown in metastatic mammary tumor cell lines. Suppression of IRS-2 in MDA-231-BO cells downregulated cell migration but not the proliferation (62). Given the ability of IRS-2 in promoting motility, this might explain why the knockdown of IRS-2 impedes metastasis of PyV-MT tumors that shown by Shaw's group (55). Collectively, both IRS-1 and IRS-2 are key signal transducers in IGF-

mediated pathway with different roles of which IRS-1 regulating proliferation and IRS-2 regulating cell migration and metastasis.

4.2) Regulation of IRSs by steroid hormones and other growth factors

Studies have shown the synergistic effect of crosstalk between ER and IGF-IR pathways in breast cancer cells. Estrogen increased IRS protein expression in MCF-7 cells and induced the tyrosine phosphorylation of both IRS-1 and IRS-2 (63,64). On the other hand, the anti-estrogens tamoxifen or ICI 182, 780 inhibited the estrogen-stimulated induction on both IRS proteins (65) and silencing IRS-1 enhanced tamoxifen-induced cell death (66). Furthermore, a variant of MCF-7 cells that lost expression of ER α (C4-12) was found to have low levels of IRSs that were restored upon re-expression of the ER (67). Other than regulating their expression, estrogen also regulates the intracellular location of IRS-1. As mentioned earlier, IRS-1 was found to interact with ER α and translocated into the nucleus as a complex upon stimulation by estrogen (30,31). Inside the nucleus, the IRS-1/ER α complex bound to the ERE of the ER α promoter and regulated transcription (30,31). Another important steroid hormone in breast cancer, progesterone, also displays a role in regulating IRS-2 (68). We found that IRS-2 mRNA and protein levels were induced by progesterone stimulation through progesterone receptor B leading to activation of the downstream signals in IGF-IR pathway and cell migration (68, 69). Taken together, these studies suggest that IRS-1 and IRS-2 mediate the crosstalk between IGF-IR and steroid hormone pathways.

Other than steroid hormones, growth factors also regulate IRS protein level and activity in breast cancer cells. Epidermal growth factor receptor (EGFR) has been shown to regulate IRS-1 expression. Indeed, EGFR associated with IRS-1 in tamoxifen-resistant MCF-7 (Tam-R) cells where it enhanced the phosphorylation of IRS-1 at serine 896 which served as the docking site for Grb2 and activated MAPK signaling (70). Such association with EGFR limited IRS-1 binding to IGF-IR and the activation of the downstream Akt, but treatment with gefitinib shifted IRS-1 back to the IGF-IR signaling. This study suggests that IRS-1 is crucial in EGFR-mediated signaling under tamoxifen-resistance. We have also shown that both IRS-1 and IRS-2 were induced by stimulation of EGF through distinct pathways (71). Elevated IRS-1 was mediated through activation of ERK1/2, whereas elevated IRS-2 was mediated through JNK.

4.3) Expression of IRSs in human breast tumors

Studies of IRS-1 expression in human breast tumors have provided inconsistent results (72, 73). In one study, IRS-1 levels were found to be strongly downregulated in Grade 3 poorly differentiated tumors (72). However, another study found that IRS-1 was expressed in Grade 3 tumors and lymph node metastasis (74). Owing to the inconsistency of IRS-1 expression in mammary tumors, the intracellular location of IRS-1 may be a more important factor in breast cancer progression. Thus, nuclear IRS-1 expression has been found to have increased frequency in breast cancer. The median frequency of nuclear IRS-1 in normal epithelium was only 1.6%, but this increased to 11% in ductal carcinoma and 35% in lobular carcinoma (75).

4.4) Prognostic significance of IRS expression

A number of studies have investigated the prognostic significance of IRS protein expression in breast cancer. Being an important component in breast cancer progression, one would predict that IRS-1 levels correlate with poor prognosis. Consistent with this, high expression of IRS-1 predicts a great incidence of recurrence after surgery (73). Also, elevated IRS-1 levels were associated with reduced disease-free survival in patients with ER-positive tumors (64,73,74). On the other hand, IRS-1 level negatively correlated with proliferation in ER negative tumors (74) and low IRS-1 expression was associated with poor prognosis (72). Interestingly, nuclear IRS-1 was found to strongly correlate with ER α in the invasive ductal breast cancer (75), and

we have found that nuclear IRS-1 is an independent predictor of outcome in response to tamoxifen therapy (Lee AV, unpublished results)

The association of single nucleotide polymorphisms (SNPs) in the IRS genes and the risk of breast cancer has recently been studied. The most common polymorphism in IRS-1 is a glycine to arginine change at codon 972 (Gly⁹⁷²Arg) (76). A study was conducted on the association between genetic variations in IGF signaling system and risk of breast cancer (77). It was found that Hispanic patients were less likely to have the A allele in IRS-1 than non-Hispanic white patients. Among post-menopausal women, the heterozygous genotype (GA) positively correlated with increased risk of breast cancer only in Hispanic women without recent exposure to hormones. Breast density is a risk factor that positively correlates with increased risk of breast cancer (78–80). In a study on the association between breast density and genetic variations in IGF signaling system, the rare AA genotype was found not to associate with breast density (81). Given that the significance of the crosstalk between the IGF and ER signaling in breast cancer, the association between the genetic variations in IRS-1 and sex hormone levels in post-menopausal breast cancer survivors was studied (82). The GA genotype was only found in African-American patients and positively correlated with estrone, testosterone and free testosterone and negatively correlated with serum hormone binding globulin. The IRS-2 Gly¹⁰⁵⁷Asp polymorphism was not found to associate with risk of breast cancer or other risk factors studied (77). However, a recent study has shown that two of three polymorphisms in IRS-2 studied are associated with increased risk of breast cancer (83). Owing to the small sample size in these studies, a conclusive statement on the association of IRS polymorphism and risk of breast cancer could not be drawn.

5) Summary

IRSs are critical elements in the IGF-IR signaling pathway and have themselves been shown to be transforming oncogenes. Studies in breast cancer cell lines and in mice have shown divergent roles for IRS-1 and IRS-2 in proliferation, migration, invasion, and metastasis. Despite all of this evidence, studies in human breast cancer are inconsistent and require further investigation. This is in part due to the paucity of studies, and also the measurement of total protein levels rather than IRS activation. Further studies using phospho-specific antibodies are likely to highlight important roles for IRSs in human breast cancer and measurement may eventually play a role in predicting response to anti-IGF-IR therapies.

Acknowledgments

We thank Dr Robert K. Dearth for reading the manuscript and giving useful comments. Dr Bonita Tak-Yee Chan is supported by the Diana Helis Henry Medical Research Foundation. Dr Adrian V. Lee's research is supported in part by grant R01CA94118 from the NIH/NCI.

Abbreviations

IRS	insulin receptor substrate
IGF-I	insulin-like growth factor I
IGF-IR	insulin-like growth factor I receptor
IR	insulin receptor
PH	plextrin homology
PTB	phosphotyrosine binding
SH2	Src-homology 2

ER	estrogen receptor
ERE	estrogen-responsive element
TEB	terminal end bud
MEF	mouse embryo fibroblast
EGFR	epidermal growth factor receptor

Literature cited

1. Lee YH, White MF. Insulin receptor substrate proteins and diabetes. *Arch Pharm Res* 2004;27(4):361–70. [PubMed: 15180298]
2. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, et al. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 1991;352(6330):73–7. [PubMed: 1648180]
3. Sun XJ, Wang LM, Zhang Y, Yenush L, Myers MG Jr, Glasheen E, et al. Role of IRS-2 in insulin and cytokine signalling. *Nature* 1995;377(6545):173–7. [PubMed: 7675087]
4. Smith-Hall J, Pons S, Patti ME, Burks DJ, Yenush L, Sun XJ, et al. The 60 kDa insulin receptor substrate functions like an IRS protein (pp60IRS3) in adipose cells. *Biochemistry* 1997;36(27):8304–10. [PubMed: 9204876]
5. Lavan BE, Fantin VR, Chang ET, Lane WS, Keller SR, Lienhard GE. A novel 160-kDa phosphotyrosine protein in insulin-treated embryonic kidney cells is a new member of the insulin receptor substrate family. *Journal of Biological Chemistry* 1997;272(34):21403–7. [PubMed: 9261155]
6. Cai D, Dhe-Paganon S, Melendez PA, Lee J, Shoelson SE. Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. *J Biol Chem* 2003;278(28):25323–30. [PubMed: 12730241]
7. Sun XJ, Miralpeix M, Myers MG Jr, Glasheen EM, Backer JM, Kahn CR, et al. Expression and function of IRS-1 in insulin signal transmission. *J Biol Chem* 1992;267(31):22662–72. [PubMed: 1385403]
8. Myers MG Jr, White MF. The new elements of insulin signaling. Insulin receptor substrate-1 and proteins with SH2 domains. *Diabetes* 1993;42(5):643–50. [PubMed: 8387037]
9. Hadsell DL, Alexeenko T, Klemintidis Y, Torres D, Lee AV. Inability of overexpressed des(1–3) human insulin-like growth factor I (IGF-I) to inhibit forced mammary gland involution is associated with decreased expression of IGF signaling molecules. *Endocrinology* 2001;142(4):1479–88. [PubMed: 11250928]
10. Wu X, Sallinen K, Anttila L, Makinen M, Luo C, Pollanen P, et al. Expression of insulin-receptor substrate-1 and -2 in ovaries from women with insulin resistance and from controls. *Fertil Steril* 2000;74(3):564–72. [PubMed: 10973656]
11. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006;7(2):85–96. [PubMed: 16493415]
12. White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 2002;283(3):E413–22. [PubMed: 12169433]
13. Bjornholm M, Zierath JR. Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. *Biochem Soc Trans* 2005;33(Pt 2):354–7. [PubMed: 15787605]
14. He W, Craparo A, Zhu Y, O'Neill TJ, Wang LM, Pierce JH, et al. Interaction of insulin receptor substrate-2 (IRS-2) with the insulin and insulin-like growth factor I receptors. Evidence for two distinct phosphotyrosine-dependent interaction domains within IRS-2. *J Biol Chem* 1996;271(20):11641–5. [PubMed: 8662806]
15. Sawka-Verhelle D, Tartare-Deckert S, White MF, Van Obberghen E. Insulin receptor substrate-2 binds to the insulin receptor through its phosphotyrosine-binding domain and through a newly identified domain comprising amino acids 591–786. *J Biol Chem* 1996;271(11):5980–3. [PubMed: 8626379]
16. Myers M, White MF. The new element of insulin signalling. *Diabetes* 1993 May;42:643–650. [PubMed: 8387037]

17. Kuhne MR, Pawson T, Lienhard GE, Feng GS. The insulin receptor substrate 1 associates with the SH2-containing phosphotyrosine phosphatase Syp. *J Biol Chem* 1993;268(16):11479–81. [PubMed: 8505282]
18. Lee CH, Li W, Nishimura R, Zhou M, Batzer AG, Myers MG Jr, et al. Nck associates with the SH2 domain-docking protein IRS-1 in insulin-stimulated cells. *Proc Natl Acad Sci U S A* 1993;90(24):11713–7. [PubMed: 8265614]
19. Amoui M, Craddock BP, Miller WT. Differential phosphorylation of IRS-1 by insulin and insulin-like growth factor I receptors in Chinese hamster ovary cells. *J Endocrinol* 2001;171(1):153–62. [PubMed: 11572799]
20. Myers MG Jr, Sun XJ, Cheatham B, Jachna BR, Glasheen EM, Backer JM, et al. IRS-1 is a common element in insulin and insulin-like growth factor-I signaling to the phosphatidylinositol 3'-kinase. *Endocrinology* 1993;132(4):1421–30. [PubMed: 8384986]
21. Ridderstrale M, Degerman E, Tornqvist H. Growth hormone stimulates the tyrosine phosphorylation of the insulin receptor substrate-1 and its association with phosphatidylinositol 3-kinase in primary adipocytes. *J Biol Chem* 1995;270(8):3471–4. [PubMed: 7876077]
22. Shepherd PR. Mechanisms regulating phosphoinositide 3-kinase signalling in insulin-sensitive tissues. *Acta Physiol Scand* 2005;183(1):3–12. [PubMed: 15654916]
23. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem J* 1998;333 (Pt 3):471–90. [PubMed: 9677303]
24. Dearth RK, Cui X, Kim HJ, Hadsell DL, Lee AV. Oncogenic transformation by the signaling adaptor proteins insulin receptor substrate (IRS)-1 and IRS-2. *Cell Cycle* 2007;6(6):705–13. [PubMed: 17374994]
25. Surmacz E. Function of the IGF-I receptor in breast cancer. *J Mammary Gland Biol Neoplasia* 2000;5(1):95–105. [PubMed: 10791772]
26. Platanias LC, Fish EN. Signaling pathways activated by interferons. *Exp Hematol* 1999;27(11):1583–92. [PubMed: 10560905]
27. White MF, Yenush L. The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol* 1998;228:179–208. [PubMed: 9401207]
28. Yamauchi T, Kaburagi Y, Ueki K, Tsuji Y, Stark GR, Kerr IM, et al. Growth hormone and prolactin stimulate tyrosine phosphorylation of insulin receptor substrate-1, -2, and -3, their association with p85 phosphatidylinositol 3-kinase (PI3-kinase), and concomitantly PI3-kinase activation via JAK2 kinase. *J Biol Chem* 1998;273(25):15719–26. [PubMed: 9624169]
29. Burfoot MS, Rogers NC, Watling D, Smith JM, Pons S, Paonessaw G, et al. Janus kinase-dependent activation of insulin receptor substrate 1 in response to interleukin-4, oncostatin M, and the interferons. *J Biol Chem* 1997;272(39):24183–90. [PubMed: 9305869]
30. Morelli C, Garofalo C, Sisci D, del Rincon S, Cascio S, Tu X, et al. Nuclear insulin receptor substrate 1 interacts with estrogen receptor alpha at ERE promoters. *Oncogene* 2004;23(45):7517–26. [PubMed: 15318176]
31. Sisci D, Morelli C, Cascio S, Lanzino M, Garofalo C, Reiss K, et al. The estrogen receptor alpha:insulin receptor substrate 1 complex in breast cancer: structure-function relationships. *Ann Oncol* 2007;(18 Suppl 6):vi81–5. [PubMed: 17591841]
32. Prisco M, Santini F, Baffa R, Liu M, Drakas R, Wu A, 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(35):32078–85. [PubMed: 12063262]
33. Chen J, Wu A, Sun H, Drakas R, Garofalo C, Cascio S, et al. Functional significance of type 1 insulin-like growth factor-mediated nuclear translocation of the insulin receptor substrate-1 and beta-catenin. *J Biol Chem* 2005;280(33):29912–20. [PubMed: 15967802]
34. Wu A, Chen J, Baserga R. Nuclear insulin receptor substrate-1 activates promoters of cell cycle progression genes. *Oncogene* 2008;27(3):397–403. [PubMed: 17700539]
35. Trojanek J, Ho T, Croul S, Wang JY, Chintapalli J, Koptyra M, et al. IRS-1-Rad51 nuclear interaction sensitizes JCV T-antigen positive medulloblastoma cells to genotoxic treatment. *Int J Cancer* 2006;119(3):539–48. [PubMed: 16572421]

36. Trojanek J, Ho T, Del Valle L, Nowicki M, Wang JY, Lassak A, et al. Role of the insulin-like growth factor I/insulin receptor substrate 1 axis in Rad51 trafficking and DNA repair by homologous recombination. *Mol Cell Biol* 2003;23(21):7510–24. [PubMed: 14559999]
37. Tu X, Batta P, Innocent N, Prisco M, Casaburi I, Belletti B, et al. Nuclear translocation of insulin receptor substrate-1 by oncogenes and Igf-I. Effect on ribosomal RNA synthesis. *J Biol Chem* 2002;277(46):44357–65. [PubMed: 12202493]
38. Sun H, Tu X, Prisco M, Wu A, Casiburi I, Baserga R. Insulin-like growth factor I receptor signaling and nuclear translocation of insulin receptor substrates 1 and 2. *Mol Endocrinol* 2003;17(3):472–86. [PubMed: 12554758]
39. Lee AV, Zhang P, Ivanova M, Bonnette S, Oesterreich S, Rosen JM, et al. Developmental and hormonal signals dramatically alter the localization and abundance of insulin receptor substrate proteins in the mammary gland. *Endocrinology* 2003;144(6):2683–94. [PubMed: 12746333]
40. Hovey RC, Trott JF, Ginsburg E, Goldhar A, Sasaki MM, Fountain SJ, et al. Transcriptional and spatiotemporal regulation of prolactin receptor mRNA and cooperativity with progesterone receptor function during ductal branch growth in the mammary gland. *Dev Dyn* 2001;222(2):192–205. [PubMed: 11668597]
41. Silberstein GB, Van Horn K, Shyamala G, Daniel CW. Progesterone receptors in the mouse mammary duct: distribution and developmental regulation. *Cell Growth Differ* 1996;7(7):945–52. [PubMed: 8809412]
42. Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, Nandi S. Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging. *J Steroid Biochem Mol Biol* 2002;80(2):137–48. [PubMed: 11897499]
43. Zeps N, Bentel JM, Papadimitriou JM, D'Antuono MF, Dawkins HJ. Estrogen receptor-negative epithelial cells in mouse mammary gland development and growth. *Differentiation* 1998;62(5):221–6. [PubMed: 9566307]
44. Lee AV, Taylor ST, Greenall J, Mills JD, Tonge DW, Zhang P, et al. Rapid induction of IGF-IR signaling in normal and tumor tissue following intravenous injection of IGF-I in mice. *Horm Metab Res* 2003;35(11–12):651–5. [PubMed: 14710342]
45. Hadsell DL, Olea W, Lawrence N, George J, Torres D, Kadowaki T, et al. Decreased lactation capacity and altered milk composition in insulin receptor substrate null mice is associated with decreased maternal body mass and reduced insulin-dependent phosphorylation of mammary Akt. *J Endocrinol* 2007;194(2):327–36. [PubMed: 17641282]
46. Heckman BM, Chakravarty G, Vargo-Gogola T, Gonzales-Rimbau M, Hadsell DL, Lee AV, et al. Crosstalk between the p190-B RhoGAP and IGF signaling pathways is required for embryonic mammary bud development. *Dev Biol* 2007;309(1):137–49. [PubMed: 17662267]
47. D'Ambrosio C, Keller SR, Morrione A, Lienhard GE, Baserga R, Surmacz E. Transforming potential of the insulin receptor substrate 1. *Cell Growth & Differentiation* 1995;6(5):557–62. [PubMed: 7647039]
48. Ito T, Sasaki Y, Wands JR. Overexpression of human insulin receptor substrate 1 induces cellular transformation with activation of mitogen-activated protein kinases. *Mol Cell Biol* 1996;16(3):943–51. [PubMed: 8622697]
49. Tanaka S, Ito T, Wands JR. Neoplastic transformation induced by insulin receptor substrate-1 overexpression requires an interaction with both Grb2 and Syp signaling molecules. *J Biol Chem* 1996;271(24):14610–6. [PubMed: 8662827]
50. Cristofanelli B, Valentinis B, Soddu S, Rizzo MG, Marchetti A, Bossi G, et al. Cooperative transformation of 32D cells by the combined expression of IRS-1 and V-Ha-Ras. *Oncogene* 2000;19(29):3245–55. [PubMed: 10918581]
51. Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* 2003;30(3):256–68. [PubMed: 12798140]
52. Dearth RK, Cui X, Kim HJ, Kuitatse I, Lawrence NA, Zhang X, et al. Mammary tumorigenesis and metastasis caused by overexpression of insulin receptor substrate 1 (IRS-1) or IRS-2. *Mol Cell Biol* 2006;26(24):9302–14. [PubMed: 17030631]

53. Aranda V, Haire T, Nolan ME, Calarco JP, Rosenberg AZ, Fawcett JP, et al. Par6-aPKC uncouples ErbB2 induced disruption of polarized epithelial organization from proliferation control. *Nat Cell Biol* 2006;8(11):1235–45. [PubMed: 17060907]
54. Ma Z, Gibson SL, Byrne MA, Zhang J, White MF, Shaw LM. Suppression of Insulin Receptor Substrate-1 (IRS-1) Promotes Mammary Tumor Metastasis. *Mol Cell Biol*. 2006
55. Nagle JA, Ma Z, Byrne MA, White MF, Shaw LM. Involvement of insulin receptor substrate 2 in mammary tumor metastasis. *Mol Cell Biol* 2004;24(22):9726–35. [PubMed: 15509777]
56. Gibson SL, Ma Z, Shaw LM. Divergent roles for IRS-1 and IRS-2 in breast cancer metastasis. *Cell Cycle* 2007;6(6):631–7. [PubMed: 17361103]
57. Nolan MK, Jankowska L, Prisco M, Xu S, Guvakova MA, Surmacz E. Differential roles of IRS-1 and SHC signaling pathways in breast cancer cells. *International Journal of Cancer* 1997;72(5):828–34.
58. Surmacz E, Burgard J-L. Overexpression of IRS-1 in the human breast cancer cell line MCF-7 induces loss of estrogen requirements for growth and transformation. *Clinical Cancer Research* 1995;1:1429–1436. [PubMed: 9815941]
59. Dalmizrak O, Wu A, Chen J, Sun H, Utama FE, Zambelli D, et al. Insulin receptor substrate-1 regulates the transformed phenotype of BT-20 human mammary cancer cells. *Cancer Res* 2007;67(5):2124–30. [PubMed: 17332342]
60. Lanzino M, Garofalo C, Morelli C, Le Pera M, Casaburi I, McPhaul MJ, et al. Insulin receptor substrate 1 modulates the transcriptional activity and the stability of androgen receptor in breast cancer cells. *Breast Cancer Res Treat*. 2008
61. Byron SA, Horwitz KB, Richer JK, Lange CA, Zhang X, Yee D. Insulin receptor substrates mediate distinct biological responses to insulin-like growth factor receptor activation in breast cancer cells. *Br J Cancer* 2006;95(9):1220–8. [PubMed: 17043687]
62. Jackson JG, Zhang X, Yoneda T, Yee D. Regulation of breast cancer cell motility by insulin receptor substrate-2 (IRS-2) in metastatic variants of human breast cancer cell lines. *Oncogene* 2001;20(50):7318–25. [PubMed: 11704861]
63. 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(17):12565–71. [PubMed: 10777546]
64. Lee AV, Jackson JG, Gooch JL, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, 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. *Molecular Endocrinology* 1999;13(5):787–96. [PubMed: 10319328]
65. Bernard L, Legay C, Adriaenssens E, Mougél A, Ricort JM. Estradiol regulates the insulin-like growth factor-I (IGF-I) signalling pathway: a crucial role of phosphatidylinositol 3-kinase (PI 3-kinase) in estrogens requirement for growth of MCF-7 human breast carcinoma cells. *Biochem Biophys Res Commun* 2006;350(4):916–21. [PubMed: 17045571]
66. Cesarone G, Garofalo C, Abrams MT, Igoucheva O, Alexeev V, Yoon K, 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(2):440–50. [PubMed: 16440325]
67. Oesterreich S, Zhang P, Guler RL, Sun X, Curran E, Welshons WV, et al. Re-expression of estrogen receptor α in estrogen receptor α -negative MCF-7 cells results in restoration of estrogen and IGF-mediated signaling and proliferation. *Cancer Research*. 2001 August 1st; In press.
68. Cui X, Lazard Z, Zhang P, Hopp TA, Lee AV. Progesterone crosstalks with insulin-like growth factor signaling in breast cancer cells via induction of insulin receptor substrate-2. *Oncogene* 2003;22(44):6937–41. [PubMed: 14534541]
69. Ibrahim YH, Byron SA, Cui X, Lee AV, Yee D. Progesterone Receptor-B Regulation of Insulin-Like Growth Factor-Stimulated Cell Migration in Breast Cancer Cells via Insulin Receptor Substrate-2. *Mol Cancer Res* 2008;6(9):1491–8. [PubMed: 18819936]
70. Knowlden JM, Jones HE, Barrow D, Gee JM, Nicholson RI, Hutcheson IR. Insulin receptor substrate-1 involvement in epidermal growth factor receptor and insulin-like growth factor receptor signalling: implication for Gefitinib ('Iressa') response and resistance. *Breast Cancer Res Treat* 2008;111(1):79–91. [PubMed: 17902048]

71. Cui X, Kim HJ, Kuitatse I, Kim H, Brown PH, Lee AV. Epidermal growth factor induces insulin receptor substrate-2 in breast cancer cells via c-Jun NH(2)-terminal kinase/activator protein-1 signaling to regulate cell migration. *Cancer Res* 2006;66(10):5304–13. [PubMed: 16707456]
72. Schnarr B, Strunz K, Ohsam J, Benner A, Wacker J, Mayer D. Down-regulation of insulin-like growth factor-I receptor and insulin receptor substrate-1 expression in advanced human breast cancer. *Int J Cancer* 2000;89(6):506–13. [PubMed: 11102895]
73. Rocha RL, Hilsenbeck SG, Jackson JG, Van Den Berg CL, Weng C-N, Lee AV, et al. Insulin-like growth factor binding protein-3 and insulin receptor substrate-1 in breast cancer: Correlation with clinical parameters and disease-free survival. *Clinical Cancer Research* 1997;3:103–109. [PubMed: 9815544]
74. Koda M, Sulkowska M, Kanczuga-Koda L, Sulkowski S. Expression of insulin receptor substrate 1 in primary breast cancer and lymph node metastases. *J Clin Pathol* 2005;58(6):645–9. [PubMed: 15917419]
75. Sisci D, Morelli C, Garofalo C, Romeo F, Morabito L, Casaburi F, et al. Expression of nuclear insulin receptor substrate 1 (IRS-1) in breast cancer. *J Clin Pathol*. 2006
76. McGettrick AJ, Feener EP, Kahn CR. Human insulin receptor substrate-1 (IRS-1) polymorphism G972R causes IRS-1 to associate with the insulin receptor and inhibit receptor autophosphorylation. *J Biol Chem* 2005;280(8):6441–6. [PubMed: 15590636]
77. Slattery ML, Sweeney C, Wolff R, Herrick J, Baumgartner K, Giuliano A, et al. Genetic variation in IGF1, IGFBP3, IRS1, IRS2 and risk of breast cancer in women living in Southwestern United States. *Breast Cancer Res Treat* 2007;104(2):197–209. [PubMed: 17051426]
78. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, et al. Mammographic breast density as an intermediate phenotype for breast cancer. *Lancet Oncol* 2005;6(10):798–808. [PubMed: 16198986]
79. Heine JJ, Malhotra P. Mammographic tissue, breast cancer risk, serial image analysis, and digital mammography. Part 2. Serial breast tissue change and related temporal influences. *Acad Radiol* 2002;9(3):317–35. [PubMed: 11887947]
80. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, et al. Heritability of mammographic density, a risk factor for breast cancer. *N Engl J Med* 2002;347(12):886–94. [PubMed: 12239257]
81. Diorio C, Brisson J, Berube S, Pollak M. Genetic polymorphisms involved in insulin-like growth factor (IGF) pathway in relation to mammographic breast density and IGF levels. *Cancer Epidemiol Biomarkers Prev* 2008;17(4):880–8. [PubMed: 18398029]
82. Fan J, McKean-Cowdin R, Bernstein L, Stanczyk FZ, Li AX, Ballard-Barbash R, et al. An association between a common variant (G972R) in the IRS-1 gene and sex hormone levels in post-menopausal breast cancer survivors. *Breast Cancer Res Treat* 2006;99(3):323–31. [PubMed: 16752222]
83. Feigelson HS, Teras LR, Diver WR, Tang W, Patel AV, Stevens VL, et al. Genetic variation in candidate obesity genes ADRB2, ADRB3, GHRL, HSD11B1, IRS1, IRS2, and SHC1 and risk for breast cancer in the Cancer Prevention Study II. *Breast Cancer Res* 2008;10(4):R57. [PubMed: 18611262]