Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Review

The role of insulin receptor substrate (IRS) proteins in oncogenic transformation

G. Gorgisen[1*], I. M. Gulacar[1], O. N. Ozes[2]

[1] Yuzuncu Yil University Medical School, Department of Medical Biology, Van, Turkey
[2] Akdeniz University Medical School, Department of Medical Biology and Genetics, Antalya, Turkey

Correspondence to: gokhangorgisen@gmail.com

Received August 19, 2016; **Accepted** January 25, 2017; **Published** January 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.1.1

Abstract: Insulin Receptor Substrate (IRS) proteins are the main cytoplasmic adaptor molecules involved in transducing extracellular signals from receptors to downstream proteins. This protein family have pivotal roles on maintenance, distribution and regulation of signaling networks. Since IRS1/2 interact with and transmits signals from the receptors of insulin, Insulin Like Growth Factor 1 (IGF1), prolactin, growth hormone (GH), leptin, Vascular Endothelial Growth Factor (VEGF), TrkB, ALK and integrins this promoted scientist to think that IRS1 may have functions in cell proliferation, tumorigenesis and metastasis. Therefore, over the past decade, studies on IRS proteins and their functions in cancer has been increased and these studies provided valuable results claiming the involvement of IRS1/2 in cancer development. In this review, we discuss the function and contributions of IRS1 and IRS2 in development of breast cancer.

Key words: Breast Cancer; Insulin Receptor Substrate Protein; Insulin Signaling; Insulin Receptor Substrate 1; Insulin Receptor Substrate 2.

Introduction

Insulin is a primary anabolic hormone responsible for maintenance of metabolic homeostasis (1,2). Insulin and insulin-like growth factors (IGF1 and IGF2) regulate anabolic and catabolic reactions through highly complex signaling networks (3). During insulin signaling, ligand-bound insulin receptor (IR) or insulin like growth factor receptor (IGF-1R) are phosphorylated at Tyrosine residues via auto-phosphorylation, then, IRS proteins bind to these phosphotyrosines through their SH2 domains. IR-bound IRS is phosphorylated by IR at YXXM motifs, four of which were localized at the C-terminal. These phosphorylated motifs create docking sites for PI3Kp85α, and YXXM-bound PI3Kp85α binds to PI3Kp110α and activates it. Activated PI3Kp110α generates PIP3 from PIP2, and therefore, activates PDK1. Activated PDK1 activates AKT by phosphorylating it at Thr308. Activated AKT regulates glucose-uptake by phosphorylating glucose transporters GLUT1 and GLUT4 (4,5). Therefore, activation of AKT is primarily responsible for the metabolic functions of insulin. IRS1/2 also activate ERK pathway which regulates the expression of genes whose products control cell proliferation and differantiation (6). (Figure 1)

In addition to being essential for glucose homeostasis, long term activations and overexpressions of IR/IGFR are strongly correlated with hiperplasia, tumor growth and poor prognosis (7-9).

In this article, we will review the recent literature, discuss the results of human and animal model studies, and explain the molecular mechanism and function of IRS proteins in development of breast cancer.

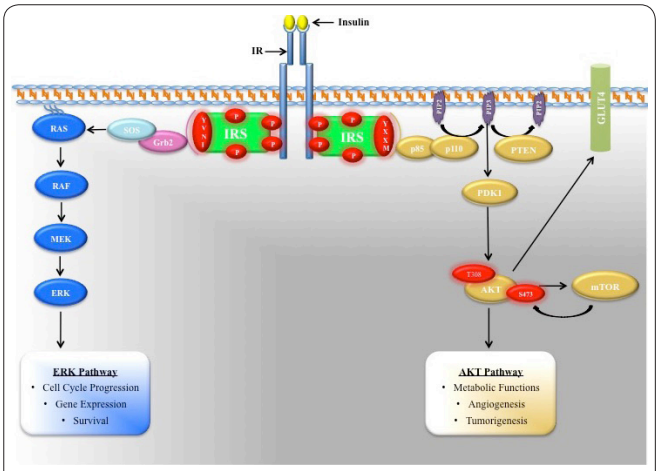

Figure 1. Schematic illustration of canonical IRS-mediated Insulin Receptor signaling pathway. IRS signaling pathway is activated by binding of insulin to insulin receptor. Tyrosine-phosphorylated IRS1 initiates both RAS and PI3K activation resulting in activation of ERK and AKT. Activated ERK and AKT further activate downstream enzymes and also directly phosphorylate several proteins to regulate metabolism as well as oncogenic transformation.

IRS proteins structure

IRS proteins are adaptor proteins and play central role in transmission of signals from multiple receptors and they elicit this through multiple phosphorylation motifs (10).

IRS family consists of four proteins (IRS1-IRS4) with two evolutionary well conserved interaction domains (2,10) (Figure 2). The first one is pleckstrin homology (PH) domain located at N-terminal of the protein and consists of positively charged aminoacids that mediates the interaction of IRS proteins with cell. This domain also leads to diversity of signaling by interac-

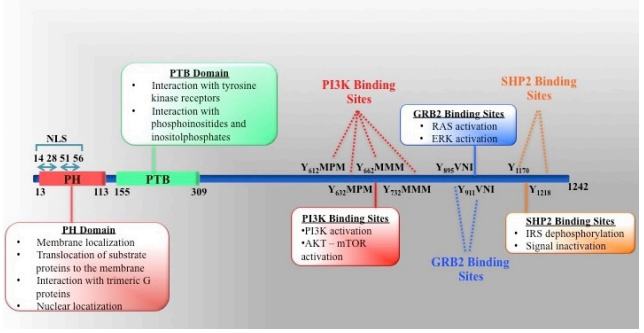

Figure 2. Schematic diagram of IRS protein family structure and interacting partners (Human IRS1): Pleckstrin homology (PH) and Phosphotyrosine Binding (PTB) domains are shown as two major functional domains in N- terminal of IRS protein family. Nuclear localization signals (NLS) of IRS1 protein are also located in PH domain. Tyrosine phosphorylation motifs to which GRB2 and SHP2 binds are also indicated in C-terminal of IRS1 protein.

ting with G-protein coupled receptors (GPCR). Nuclear localization signal of IRS1 protein is also located in this region (11-13).

The second conserved region is phosphotyrosine binding (PTB) domain. This domain form a pocket which binds to phosphorylated NPXY motifs of activated insulin receptor. IRS2 has an additional motif that is called the kinase regulatory loop binding domain (KRLB), which contribute to receptor recruitment (10,14).

The carboxy terminus of IRS proteins are poorly conserved. They contain multiple putative tyrosine phosphorylation sites which function as on/off switches of signaling and provide docking sites for Src homology 2 domain (SH2) containing proteins such as p85α subunit of phosphatidiylinositol 3 kinase, Grb2, Crk, Csk, Fyn, Nck, PLCγ and SHP2 (15,16). In addition to tyrosine phosphorylation motifs, more than 200 Ser/Thr (S/T) residues are located in this region and many of them are recognized by various S/T kinases. These phosphorylation sites contribute unique specificity owing to unique regulations of IRS proteins. Unlike tyrosine phosphorylations, S/T phosphorylations generally negatively regulate insulin /IGF signaling (1,17).

Expressions and biological functions of IRS proteins

IRS1 is the first identified member of this family as IR substrate. This gene is located on human chromosome 2q34-37 and encoded by single exon. It is widely expressed in human tissues. *IRS2* gene located at 13q34 chromosome region contains two exons and its product has been ubiquitously expressed in human tissues. IRS3 is expressed only in rat not in human. Unlike IRS1 and IRS2 proteins, IRS4 has restricted expression pattern and is found primarily in liver, kidney, thymus and brain (18,19). Owing to their expression profiles, most of the studies focus on IRS1 and IRS2 proteins and their functions (9,20).

Studies on *IRS* knock-out animal models provided strong evidences on biological roles of IRS proteins. *IRS1*-/- mice are born 30% smaller than wild type (WT) mice and growth is reduced throughout their lives. These models also have insulin resistance and hyperinsulinemia but they do not develop diabetes and maintaine normal pancreatic beta cell numbers (21,22). In contrast to *IRS1* -/- model, *IRS2* -/- mice have normal size and develop early onset diabetes with decreased beta cell mass. Neuronal proliferation is also decreased in these models and females are infertile (23-25). These phenotypes showed that IRS1 and IRS2 have non-redundant roles even they share significant homology. *IRS4* -/- mice are phenotypically normal but have moderate growth retardation, insulin sensitivity defects and reproductive problems (26).

These differences in *IRS* -/- models especially in somatic growth are also evident in cancer. In addition to these physiological functions of IRS proteins, their interactions with growth factor/hormone receptors such as IGFR, leptin, vascular endothelial growth factors (VEGF), growth hormone, prolactin, integrin, cytokine and interferon receptors in cellular level shows the pivotal roles of IRS proteins in cell proliferation, tumorigenesis and metastasis (16,27-32)(Figure 3).

The roles of IRS1 and IRS2 proteins in developing breast cancer

IRSs expressions in breast cancer cell lines

IGF system has an important role in mammary gland development, and several studies have shown the critical roles of IRS1 and IRS2 in normal mammary gland development (33-35). In addition to this, several studies have shown critical roles of IRS1 and IRS2 in development of breast cancer. Transformation capacity of IRS1 protein was first shown Surmaczs' group in mouse embryo fibrablasts (MEFs) (36). Dearth et al. demonstrated that IRS-mediated transformation of mammary epithelial cells in nontransformed human mammary epithelial cells (MCF10A). In this study, they overexpressed IRS1 and IRS2 proteins in MCF10A cells these over-expressions induced proliferations and disrupted the polarity of cells grown in 3D culture (37).

In MCF-7 cell lines, suppression of IRS-1 by siRNA strongly inhibited anchorage-dependent and -independent cell growth and induce apoptotic cell death

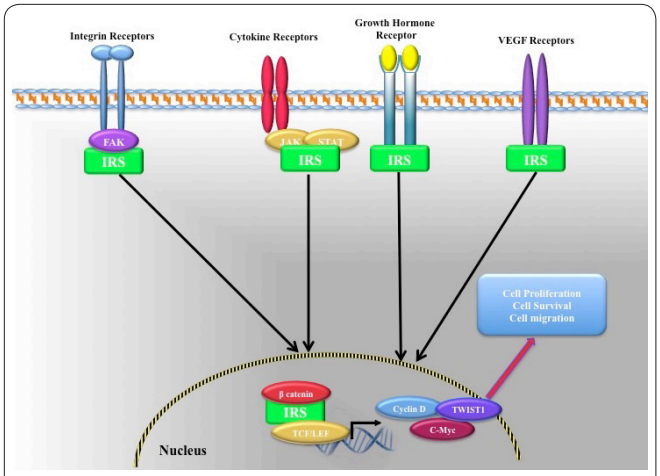

Figure 1. Schematic illustration of noncanonical IRS signaling mentioned in this review. IRS proteins can interact with ligand-bound receptors cytokines, growth hormone (GH), growth factors and integrin receptors. Activated IRS proteins translocate to nucleus and bind to β-catenin and activate transcription of several β-catenin target genes such as c-myc, twist1, and cyclin D1. pathways.

under growth factor- and estrogen reduced conditions (38). On the other hand, MCF7 cells overexpressing IRS1 do not need estrogen for growth and their estrogen requirements were decreased gradually with the level of IRS1 overexpression. Recently a study showed that, knockdown of IRS1 expression by siRNA enhanced tamoxifen induced cell death in MCF7 (39). IRS1 overexpression also promotes growth and proliferation of BT 20 cells, and induced formation of larger tumors in *in vivo* (40). IRS proteins may also be used as prognostic factor in breast cancer. Becker et al. ectopically expressed IRS1 or IRS2 in IRS-null T47D-YA cells and determined their proliferation, motility and global gene expression profile. They also found that IRS1/2 play significant role in induction of TGFβ2, which was claimed to be responsible for IGF1-mediated cell migration. In addition, they also determined level of IRS1 expression in luminal B breast cancer type and found that high expression of IRS1 was correlated with poor relapse-free and overall survival, therefore, they suggested that IRS1 expression could be used as marker to predict response to anti IGF-therapy in breast cancer (41).

IRSs expressions in in vivo studies

To determine the roles of IRS1 and IRS2 proteins in tumor initiation and progression *in vivo*, researchers generated knock out and transgenic mice with mammary specific overexpression MMTV-HA-IRS1 and MMTV-HA-IRS2 (37). In mice overexpressing IRS1 and IRS2 preneoplastic mammary hyperplasia developed at 24 weeks of age. Multiple mammary gland lesions appeared at 92 weeks in HA-IRS1 overexpressed mice while overexpression of IRS2 caused more rapid tumor formation with a mean time of 68 weeks, these results suggested that multiple and different signaling pathways and oncogenes could be activated by IRS1 and IRS2. In addition to their transformation capacity, overexpressions of IRS1 and IRS2 were detected in 33% and 40% of metastatic lung tumors, respectively (37).

To clarify the molecular mechanisms used by IRS proteins in mammary tumorigenesis, Dr. Dearth et all examined tyrosine phosphorylation of IRS1/2 levels and the activation of downstream elements and found that IRS1/2 were constitutively phosphorylated in mice overexpressing both IRS proteins. Moreover, while ERK1/2 phosphorylation was increased in all tumor cells, phosphorylation AKT was observed in tumors only 10% of animals after infusion of IGF1. These results clearly indicate that activation of ERK and AKT by IRS1/2 is not mediated by a common pathway. This study also showed that in both transgenic mice IRS1/2 interact with and activate β-catenin resulting in induction of expression β-catenin target such as c-myc and cyclin D1 (37) (Figure 3).

In another group of studies Shaw et al. generated polyoma virus middle T:IRS1 null and IRS2 null transgenic mice to identify the specific roles of IRS1/2 in tumor progression and metastases (42-44). Both group of animals developed tumors with the same onset and growth rate, however, unlike the redundant roles of IRS1/2 in tumor growth, they have distinct effects on tumor progression. IRS1 -/- mice showed increase in lung metastases while lung metastases decreased in IRS2 -/- mice. They also showed that ectopic expression of IRS2 compensated the loss of IRS1 in tumor metastases by increasing phosphorylations and activations of AKT/mTOR, but they did not observe IRS1 compensation in IRS2 -/- mice (43). These results suggested that IRS2 is primarily responsible for cell motility and metastases while IRS1 is mainly important for cell proliferation (42-44).

IRS expressions in human breast tumors

After establishment of convincing publications about involvement of IRS1/2 in breast cancer development and metastasis several groups determined expression levels of IRS1 and IRS2 in human breast cancer samples by immunohistochemistry, however, results of these studies produced controversial results. For example, Schnarr et. Al. found that both IGFR and IRS1 expressions were down-regulated in advanced human breast cancer cells whereas Koda et al showed that IRS1 expression was elevated in human breast cancer cells and its lymph node metastases (45,46). Porter et al. also analyzed IRS1 and IRS2 expression in human breast tumor samples and found that IRS1 expression was elevated in localized breast tumors while IRS2 expression was associated with an invasive breast tumors (47). These results are in good agreement with previous publications (42-44) which claimed induction and supression of breast cancer cells by IRS2 and IRS1, respectively.

Conclusion

IRSs are critical elements and multitask proteins that interacts with a multitude of proteins in tumorigenesis. Although several publications determined expression levels of IRS1 and IRS2 in different type and models of breast cancer samples, we don't know much about phosphorylation status of IRS1 and IRS2. Also, we don't know how IRS proteins are localized in different type of breast cancer cells, and whether they use different partners to elicit their functions. In addition to these, it is important to determine the levels of phosphatases which dephosphorylate IRS proteins. Therefore, in addition to expression analysis component of signaling molecules which directly affect modifications and functions of IRS1 and IRS2 must be determined in tumor samples simultaneously.

References

1. Herschkovitz A, Liu YF, Ilan E, Ronen D, Boura-Halfon S, Zick Y. Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. The Journal of Biological Chemistry 2007; 282: 18018-18027.

2. Schmitz-Peiffer C, Whitehead JP. IRS-1 regulation in health and disease. IUBMB Life 2003; 55: 367-374.

3. DeBosch BJ, Muslin AJ. Insulin signaling pathways and cardiac growth. Journal of Molecular and Cellular Cardiology 2008; 44: 855-864.

4. Bevan P. Insulin signalling. Journal of Cell Science 2001; 114: 1429-1430.

5. Wing SS. The UPS in diabetes and obesity. BMC Biochemistry 2008; 9 Suppl 1: S6.

6. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nature Reviews. Molecular Cell Biology 2006; 7: 85-96.

7. Denduluri SK, Idowu O, Wang Z, Liao Z, Yan Z, Mohammed MK, et al. Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. Genes & Diseases 2015; 2: 13-25.
8. Sachdev D, Yee D. Disrupting insulin-like growth factor signaling as a potential cancer therapy. Molecular Cancer Therapeutics 2007; 6: 1-12.
9. Mardilovich K, Pankratz SL, Shaw LM. Expression and function of the insulin receptor substrate proteins in cancer. Cell Communication and Signaling : CCS 2009; 7: 14.
10. White MF. IRS proteins and the common path to diabetes. American journal of physiology. Endocrinology and Metabolism 2002; 283: E413-422.
11. Nawaratne R, Gray A, Jorgensen CH, Downes CP, Siddle K, Sethi JK. Regulation of insulin receptor substrate 1 pleckstrin homology domain by protein kinase C: role of serine 24 phosphorylation. Molecular Endocrinology 2006; 20: 1838-1852.
12. 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. The Journal of Biological Chemistry 2002; 277: 32078-32085.
13. Reiss K, Del Valle L, Lassak A, Trojanek J. Nuclear IRS-1 and cancer. Journal of Cellular Physiology 2012; 227: 2992-3000.
14. Eck MJ, Dhe-Paganon S, Trub T, Nolte RT, Shoelson SE. Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. Cell 1996; 85: 695-705.
15. Porter HA, Carey GB, Keegan AD. Insulin receptor substrate 1 expression enhances the sensitivity of 32D cells to chemotherapy-induced cell death. Experimental Cell Research 2012; 318: 1745-1758.
16. Shaw LM. Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the alpha6beta4 integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion. Molecular and cCellular Biology 2001; 21: 5082-5093.
17. Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. Diabetologia 2012; 55: 2565-2582.
18. Fantin VR, Sparling, JD, Slot JW, Keller SR, Lienhard GE, Lavan BE. Characterization of insulin receptor substrate 4 in human embryonic kidney 293 cells. The Journal of Biological Chemistry 1998; 273: 10726-10732.
19. Lee YH, White MF. Insulin receptor substrate proteins and diabetes. Archives of Pharmacal Research 2004; 27: 361-370.
20. Hanke S, Mann M. The phosphotyrosine interactome of the insulin receptor family and its substrates IRS-1 and IRS-2. Molecular & Cellular Proteomics : MCP 2009; 8: 519-534.
21. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, 3rd Johnson RS., et al. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 1994; 372: 186-190.
22. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, et al. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 1994; 372: 182-186.
23. Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, Flint CL, et al. Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 2003; 23: 7084-7092.
24. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nature Genetics 1999; 23: 32-40.
25. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998; 391: 900-904.
26. Fantin VR, Wang Q, Lienhard GE, Keller SR. Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. American journal of physiology. Endocrinology and Metabolism 2000; 278: E127-133.
27. Argetsinger LS, Hsu GW, Myers MG, Jr Billestrup N, White MF, Carter-Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. The Journal of Biological Chemistry 1995; 270: 14685-14692.
28. Bjorbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. The Journal of Biological Chemistry 1997; 272: 32686-32695.
29. Johnston JA, Wang LM, Hanson EP, Sun XJ, White MF, Oakes SA, et al. Interleukins 2, 4, 7, and 15 stimulate tyrosine phosphorylation of insulin receptor substrates 1 and 2 in T cells. Potential role of JAK kinases. The Journal of Biological Chemistry 1995; 270: 28527-28530.
30. Platanias LC, Uddin S, Yetter A, Sun XJ, White MF. The type I interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2. The Journal of Biological Chemistry 1996; 271: 278-282.
31. Senthil D, Ghosh Choudhury G, Bhandari BK, Kasinath BS. The type 2 vascular endothelial growth factor receptor recruits insulin receptor substrate-1 in its signalling pathway. The Biochemical Journal 2002; 368: 49-56.
32. Vuori K, Ruoslahti E. Association of insulin receptor substrate-1 with integrins. Science 1994; 266: 1576-1578.
33. Hovey RC, Harris J, Hadsell DL, Lee AV, Ormandy CJ, Vonderhaar BK. Local insulin-like growth factor-II mediates prolactin-induced mammary gland development. Molecular Endocrinology 2003; 17: 460-471.
34. 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. Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2003; 35: 651-655.
35. 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: 2683-2694.
36. D'Ambrosio C, Keller SR, Morrione A, Lienhard GE, Baserga R, Surmacz E. Transforming potential of the insulin receptor substrate 1. Cell growth & differentiation : The Molecular Biology Journal of the American Association for Cancer Research 1995; 6: 557-562.
37. Dearth RK, Cui X, Kim HJ, Kuiatse I, Lawrence NA, Zhang X, et al. Mammary tumorigenesis and metastasis caused by overexpression of insulin receptor substrate 1 (IRS-1) or IRS-2. Molecular and Cellular Biology 2006; 26: 9302-9314.
38. 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. Int J Cancer 1997; 72(5): 828-34.
39. Surmacz E, Burgaud JL. Overexpression of insulin receptor substrate 1 (IRS-1) in the human breast cancer cell line MCF-7 induces loss of estrogen requirements for growth and transformation. Clin Cancer Res 1995; 1(11): 1429-36.
40. 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. Clin Cancer Res 2007; 67(5): 2124-30.
41. Becker MA, Ibrahim YH, Oh AS, Fagan DH, Byron AS, Sarver AL. Insulin Receptor Substrate Adaptor Proteins Mediate Prognostic Gene Expression Profiles in Breast Cancer. Plos One 2016;11(3):1-12.

42. Gibson SL, Ma Z, Shaw LM. Divergent roles for IRS-1 and IRS-2 in breast cancer metastasis. Cell Cycle 2007; 6: 631-637.
43. Ma Z, Gibson SL, Byrne MA, Zhang J, White MF, Shaw LM. Suppression of insulin receptor substrate 1 (IRS-1) promotes mammary tumor metastasis. Molecular and Cellular Biology 2006; 26: 9338-9351.
44. Nagle JA, Ma Z, Byrne MA, White MF, Shaw LM. Involvement of insulin receptor substrate 2 in mammary tumor metastasis. Molecular and Cellular Biology 2004; 24: 9726-9735.
45. 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. International Journal of Cancer 2000; 89: 506-513.
46. Koda M, Sulkowska M, Kanczuga-Koda L, Sulkowski S. Expression of insulin receptor substrate 1 in primary breast cancer and lymph node metastases. Journal of Clinical Pathology 2005; 58: 645-649.
47. Porter HA, Perry A, Kingsley C, Tran NL, Keegan AD. IRS1 is highly expressed in localized breast tumors and regulates the sensitivity of breast cancer cells to chemotherapy, while IRS2 is highly expressed in invasive breast tumors. Cancer Letters 2013; 338: 239-248.