Indian Journal of Biochemistry & Biophysics
Vol. 51, December 2014, pp. 493-498

Review

Insulin Signaling Network in Cancer

Alpana Ray, Mohamed Alalem and Bimal K Ray*

Department of Veterinary Pathobiology, University of Missouri, Columbia, Missouri, USA

Received 14 August 2014; *revised 20 November 2014*

The primary function of insulin is viewed as a hormone that controls blood glucose level. However, there is growing evidence that aberrant insulin level and insulin-mediated signaling can lead to cancer development and progression. The insulin-cancer relationship has stemmed from various observational and epidemiological studies, which linked higher incidence of cancer with central obesity, type II diabetes and other conditions associated with increased levels of circulating insulin, insulin resistance and hyperinsulinemic states. Increased risk of developing a range of cancers is also seen with a certain treatment options used to lower blood glucose level in diabetic patients. While metformin monotherapy has the lowest risk of developing cancer, in comparison, treatment with insulin or insulin secretagogues shows more likelihood to develop solid cancers. Cellular signaling initiated by insulin provides a clue regarding these diverse cellular outcomes. This review discusses how the insulin enacts such diverse physiological effects and the insulin-cancer relationship, with focus on the role of insulin signaling in cancer.

Keywords: Insulin, Insulin-like growth factors, Insulin receptors, IGF-R, Insulin-like growth factor receptor, Insulin receptor substrate, PI3 kinase, Akt, GLUT4, mTOR

Introduction

In the fall of 1921, Fredrick Banting and Charles Best discovered that extract prepared from pancreas can dramatically lower the high blood sugar level in depancreatized, diabetic dogs. Following this discovery, both Banting and Best along with James Collip, all working in the laboratory of John Macleod, purified an active ingredient called insulin. In early 1922, a human clinical trial of insulin on patients with diabetes mellitus showed great success. The discovery of insulin was announced on May 3, 1922 by Macleod at a meeting of the Association of American Physicians. For their pioneering work on insulin, Banting and Macleod received the 1923 Nobel Prize in the area of Medicine/Physiology. In his Nobel Award accepting speech, Dr. Banting said "insulin is not a cure for diabetes, it is a treatment". Interestingly, more than 90 years later, insulin still remains as a major treatment modality for diabetes. While the beneficial role of insulin in diabetes is well-recognized, enhanced level of insulin-mediated signaling could have deleterious consequences. A case in point is the harmful effect of insulin in cancer growth. Cellular function of insulin provides a clue for its harmful effect in cancer, where it is present at a higher level and highly active in mediating its function[1,2].

How does insulin function?

Insulin allows most cells to absorb glucose from circulating blood and thereby it permits utilization of glucose for producing energy[3]. Physiological level of insulin is critical for maintaining adequate, not very high or very low levels of blood sugar. Insulin level rises on demand when blood sugar level is increased, following consumption of a meal. The signal of high glucose in the blood stream initiates insulin secretion from the pancreatic β cells, where it is synthesized and remains stored. By interacting with transmembrane insulin receptor (IR) proteins, insulin alters the permeability of cell membrane to glucose and promotes glucose adsorption. Insulin receptor is encoded by a single gene from which two isoforms of insulin receptor, IR-A and IR-B are generated via

*Corresponding author
E-mail: rayb@missouri.edu
Tel: 01-573-882-4461; Fax: 01-573-884-5414

Abbreviations: AMPK, cyclic AMP-dependent kinase; eIF4EBP1, eIF4E-binding protein 1; GLUT4, glucose transporter 4; IGF, insulin-like growth factor; IGF-BP, IGF-binding protein; IGF-R, insulin-like growth factor receptor; IR, insulin receptor; IRS-1, insulin receptor substrate-1; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PDK1, 3-phosphoinositide dependent protein kinase-1; PI3K, phosphoinositydyl 3-OH kinase; PIP3, phosphatidylinositol (3, 4, 5)-trisphosphate; Rheb, Ras homologue enriched in brain; S6K, ribosomal S6 kinase; TSC, tuberous sclerosis complex.

alternative splicing. In IR-A the exon 11, which codes for 12 amino acids is excluded and in IR-B, the exon 11 is included. Both, IR-A and IR-B proteins undergo further post-translational modifications and then assemble as homo- or hetero-dimer molecules to form the functional insulin receptor protein. When glucose enters the bloodstream, pancreas secretes insulin, which then binds to the dimerized insulin receptors and initiate intracellular signaling. Interaction of insulin with insulin receptor activates an intrinsic tyrosine protein kinase that is present in the intracellular domain of the IR. Activated insulin receptor kinase phosphorylates and activates a number of intracellular glucose transporter proteins[4], which upon assembly to the cell membrane open a channel, so that glucose can enter the cell. The glucose transporter proteins although are constitutively expressed and present in the cell, without the insulin-mediated signaling, these proteins remain entrapped within the intracellular cytoplasmic vesicles and cannot transport any glucose. The insulin-IR mediated signaling allows rapid movement of the cytoplasmic vesicles to the cell membrane, where the cytoplasmic vesicles fuse and allow entry of the glucose transporters into the cell membrane (Fig. 1). When blood glucose level declines, insulin no longer binds to the insulin receptors and the glucose transporters recycle back into the cytoplasm. Thus, the big picture is that insulin through its action of signal transduction controls the use of cellular fuel like sugar, for producing energy and imparts a profound effect on cellular homeostasis. Dysregulation of the insulin-signaling pathways, consequently would lead to widespread and devastating effects on many organs and tissues. In this review, we will focus the impact of insulin-signaling pathways in cancer.

Cancer cells utilize insulin to meet their high appetite for energy

From cohort studies, a direct link between high level of insulin and increased risk of breast cancer [5] and cancers of the pancreas, colorectal, lung and colon became evident[6-9]. Insulin has been shown to stimulate growth of breast cancer cells[10-12] and in a non-obese mouse model of type II diabetes, it accelerates breast cancer development and progression[13]. The mechanisms by which insulin promotes cancer are yet to be fully resolved, but it can be speculated that cancer cells which have much higher growth rate require higher level of energy source and high glucose uptake, which obviously could be met with increased supply of insulin and insulin receptors or both. Further information on the insulin-cancer relationship can be found in a recent review[14].

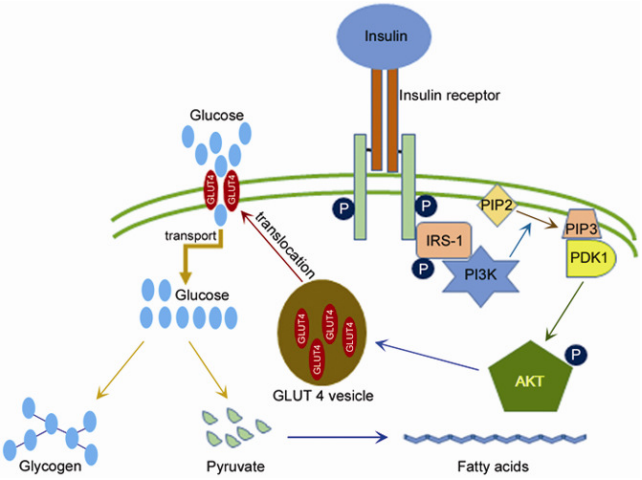

Fig. 1—A model depicting mechanism of action of insulin for glucose transport inside a cell [Upon binding of insulin to the dimerized insulin receptor, phosphorylation of insulin receptor occurs, which allows recruitment of insulin receptor substrate-1 (IRS-1) to the plasma membrane-bound insulin receptor. IRS-1 recruits phosphoinositydyl 3-OH kinase (PI3K) which subsequently activates 3-phosphoinositide dependent protein kinase-1 (PDK1) by allowing PDK1 interaction with phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3). PDK1 in turn activates AKT, also known as protein kinase B. Akt allows glucose transporter 4 (GLUT 4) to translocate to the plasma membrane. GLUT-4 facilitates the movement of glucose from outside of the cells to inside. Once inside the cell and located in the cytoplasm, glucose is either stored as glycogen, or converted to pyruvate via glycolysis and produce energy. Pyruvate is also converted to fatty acids for storage and future utilization]

Role of insulin receptors in cancer

There is now clear evidence that in breast cancer cells, insulin promotes tumor growth and enhances tumor progression by acting through significantly over-expressed insulin receptors[14,15]. Although insulin receptors are widely distributed, most normal cells contain very few of these proteins. The reason for low abundance of insulin receptor has been attributed to rapid turnover of this molecule. In normal human lymphocytes, insulin has been shown to accelerate degradation of insulin receptor proteins[16]. Consistent with this finding, insulin treatment in diabetic rats is found to reduce the level of IR mRNA[17,18]. These

findings suggest that under normal condition, insulin down-regulates the level of insulin receptors and insulin most likely regulates IR gene expression by transcriptional, post-transcriptional, translational and/or post-translational regulatory processes, which alone or in combination are impaired in cancer cells. However, the results on the effect of insulin on IR mRNA level are ambiguous, probably due to the studies conducted in different cell types[19-23], including a recent observation rat hepatoma cells, indicating that although insulin reduces IR protein level, it is not involved in the inhibition of IR gene expression[24].

As mentioned earlier, concentrations of functional insulin receptors and insulin stimulated insulin receptor tyrosine kinase activity are markedly elevated in breast cancer tissues and cells[15]. Pivotal role of insulin receptors in cancer is further supported by the finding that over-expression of the IRs in fibroblast and ovary cells induces a ligand-mediated transformed phenotype [25]. Overexpression of IR has been found to be essential for virus-induced tumorigenesis of Kaposi's sarcoma[26]. Higher level of IR is found to be linked with poor prognosis in breast cancer[27,28]. Although both IR-A and IR-B receptors participate in the insulin-mediated signaling, IR-A is more commonly over-expressed in cancer and thereby appears to play a higher role (reviewed earlier[14]). The molecular mechanisms whereby insulin receptors promote cancer are still not clear, but studies have shown that breast cancer cells utilize insulin more efficiently due to an increased sensitivity of insulin receptors for the hormone[29,30]. Such an increased sensitivity has been attributed to tumor-associated resistance to down-regulation of insulin receptor by insulin.

Involvement of insulin-like growth factor and its receptor in cancer

Insulin-like growth factors (IGFs) represent a group of proteins that have high sequence similarity to insulin and similar functional properties. IGFs contain two main members, IGF-1 and IGF-2. While insulin is synthesized by the pancreatic β cells, IGFs are mainly synthesized by the liver under the control of growth hormone (GH), but insulin is also one of the regulators of liver IGF-1 production. IGFs function through interaction with two IGF receptors (IGF-IR and IGF-IIR) and a group of IGF-binding proteins (IGFBPs). The type I IGF receptor (IGF-IR) is a tyrosine kinase, which is closely related to the insulin receptor (IR). IGF-IR can form heterodimer with insulin receptors, IR-A and IR-B and similar to IR, IGF-IR mediates its signal through pI3K and Akt signaling pathway and regulates both metabolic and non-metabolic functions (reviewed earlier[14]). In contrast to IGF-IR, the type II IGF receptor (IGF-IIR) lacks a tyrosine kinase activity and therefore does not transduce signals. One of the prominent functions of IGF-IIR is to remove IGF-2 from the cell surface and attenuate IGF-2 mediated cell signaling. After binding to IGF-2, the IGF-2/IGF-IIR complex gets accumulated in the vesicles, which then get internalized.

The bioavailability of IGF proteins is also regulated by the IGFBPs, which serve as carrier proteins for IGF-1. Almost all, about 98% of IGF-1 always remains bound to one of the six IGFBPs, but IGFBP-3 accounts for the most IGF-1 sequestration. Insulin, which promotes IGF-1 synthesis via growth hormone can also increase the quantity of bioavailable IGF-1 by decreasing the levels of IGFBP-3 and IGFBP-1. Lower levels of these IGFBPs result in more unbound IGF-1 that becomes free to interact with the IGF-1 receptor (IGF-IR). The IRs, IGFs, IGFRs and IGFBPs are part of an intricate regulatory system in insulin-signaling that is critical in the maintenance of cellular homeostasis and perturbation of the orchestrated interplay of these molecules can lead to abnormalities in both metabolic and non-metabolic functions[14].

Tumor cells are found to overexpress IGF-1 and dysregulation of IGF-mediated signaling pathways, plays a critical role in cancer. The role of IGF-1 in cancer has become increasingly clear from the studies in patients with the acromegaly syndrome that results in secretion of higher level of growth hormone (GH) and IGF-1 synthesis; these patients have exhibited increased risks of colon, thyroid, prostate and breast cancer[31]. A strong link between higher circulating levels of IGF-1 and increased risks of breast and colorectal cancers is further supported from independent cohort studies[32,33]. In correlation, serum level of IGF-IR is found to be significantly higher in those patients with short-time (less than 5 yrs) recurrence-free and overall low survival rates, as compared to those patients with low-level expression of IGF-IR[34]. In animal models, overexpression of IGF-1 is shown to promote tumor growth[35], while IGF-1 deficiency decreases risk of mammary and colon cancers[36,37]. Consistent with the implication of IGF-1 system as a risk factor, low level of IGF is shown to be linked with the reduced growth of cancer

cells[38]. Like IGF-1, IGF-2 is overexpressed in certain cancers and has an adverse effect in disease prognosis. In a cohort study of esophageal carcinoma, high level expression of IGF-2 is found to be associated with reduced disease-free survival[39].

Role of insulin-regulated mTOR signaling in cancer

The mammalian target of rapamycin (mTOR) is classified as a master regulator which integrates the signals from nutrient and energy sensors with cell growth and proliferation and thereby plays a central role in the regulation of cell growth, proliferation, differentiation, migration and survival[40-42]. The mTOR protein is a serine threonine protein kinase, which itself gets activated by phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt/PKB). Thus, insulin, IGF receptors and other growth factors that can activate the PI3K/Akt signaling pathway are able to activate mTOR, formation of an active mTOR complex (mTORC1) and mTOR-mediated signaling (outlined in Fig. 2). Upon activation, mTOR phosphorylates at least two translational components, ribosomal S6 Kinase (S6K) and eIF-4E binding protein (eIF4EBP) and thereby promotes cellular protein synthesis. It is worthy of mention that the mechanisms by which nutrients and ATP activate mTOR are still not fully known.

Dysregulation of the mTOR signaling pathway, which can create a favorable environment for growth is reported in many human cancers[43,44]. Additionally, changes in the level of the components of the mTOR signaling pathway have been reported in cancers of breast, prostate, pancreas, ovary, lung and lymphoma[43,45-51]. In endometrial cancer, high level of the phosphorylated form of mTOR is reported[52,53]. The evidences suggesting significant dysregulation of the mTOR signaling pathway in cancer have raised the possibility of using mTOR inhibitors for cancer therapy. Rapamycin and its derivatives are classical inhibitors of mTOR; these agents by successful inhibition of S6K and eIF-4E can lead to cell growth inhibition and activation of apoptosis. A number of studies using inhibitors of the mTOR signaling pathway are currently underway and recent preclinical and clinical evidences suggest that reduction of mTOR function might be effective in anti-cancer therapy[54-57].

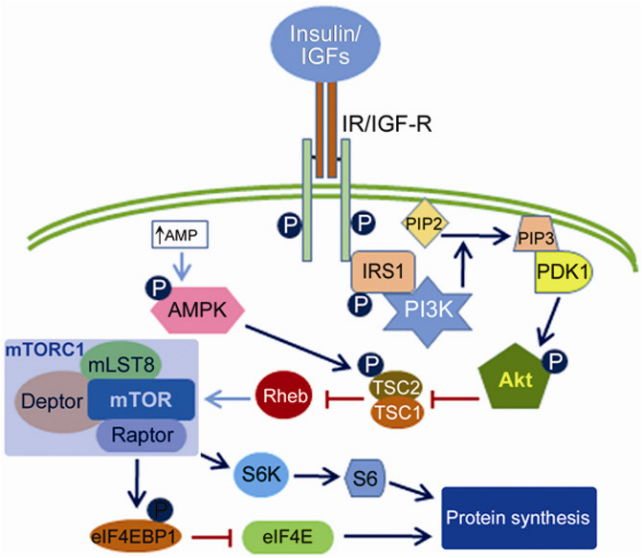

Fig. 2—A model depicting insulin and insulin-like growth factor mediated cell growth via increased protein synthesis [Following ligand-mediated activation of receptors (IR/IGF-R), a series of activation events leads to phosphorylation and subsequent activation of Akt. Activated Akt, in turn, phosphorylates TSC (tuberous sclerosis complex) proteins, TSC1 and TSC2. The TSC1-TSC2 (hamartin-tuberin) complex through its GAP (GTPase-activating protein) activity towards the small G-protein Rheb (Ras homologue enriched in brain), negatively regulates mTORC1 (mammalian target of rapamycin complex 1) activity. Phosphorylation of TSC1/2 complex allows activation of mTOR in the mTORC1 complex, which subsequently phosphorylates eIF4E-binding protein 1 (eIF4EBP1) and ribosomal S6 kinase (S6K). Phosphorylation of eIF4EBP1 allows this protein to be dissociated from eIF4E which is an essential translational initiation factor. Once released from the inhibitory complex of eIF4EBP1/eIF4E, eIF4E becomes accessible for initiation of protein synthesis. S6 kinase on the other hand is involved in activating ribosomal protein S6 which is a component of 40S ribosomal subunit. S6 kinase has several other substrates suggesting multiple levels at which mTORC1-S6K pathway regulates cellular processes. Cyclic AMP-dependent kinase (AMPK), when activated by increased availability of cAMP also activates mTOR and subsequent signaling pathways for increased protein synthesis and growth]

Conclusion

The association between type II diabetes, the choice of insulin therapy and the risk of cancer has hitherto attracted the attention on the role of insulin-mediated signaling in cancer development and progression. Many details of insulin-mediated signaling are still unknown, but it is clear that intricately complex cellular regulation mediated by insulin and its receptors, IGFs, IGF receptors and IGF binding proteins plays a critical role in maintaining normal homeostasis. Abnormal activity and/or aberrant expression of any of the key components of

this pathway will create not only the risk of type II diabetes, but also cancer. Further understanding on insulin-mediated signaling and development of potential anti-cancer therapies by blocking this signaling pathway is a future clinical possibility.

Acknowledgements

We are grateful for support from US Army Medical Research and Material Command grant W81XWH-09-1-0084 and University of Missouri Research Council grant.

References

1 Pollak M (2008) Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 8, 915–928
2 Boyd DB (2003) Insulin and cancer. *Integr Cancer Ther* 2, 315–329
3 Aronoff S L, Berkowitz K, Shreiner B & Want L (2004) Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum* 17, 183-190
4 Olson A L (2012) Regulation of GLUT4 and Insulin-Dependent Glucose Flux. *ISRN Molecular Biology* 2012, Article ID 856987, 12 pages
5 Goodwin P J, Ennis M, Pritchard K I, Trudeau M E, Koo J, Madarnas Y, Hartwick W, Hoffman B & Hood N (2002) Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol* 20, 42–51
6 Michaud D S, Liu S, Giovannucci E, Willett W C, Colditz G A & Fuchs C S (2002) Dietary sugar, glycemic load, and pancreatic cancer risk in a prospective study. *J Natl Cancer Inst* 94,1293-1300
7 Franceschi S, Dal Maso L, Augustin L, Negri E, Parpinel M, Boyle P, Jenkins D J & La Vecchia C (2001) Dietary glycemic load and colorectal cancer risk. *Ann Oncol* 12, 173-178
8 De Stefani E, Deneo-Pellegrini H, Mendilaharsu M, Ronco A & Carzoglio J C (1998) Dietary sugar and lung cancer: a case-control study in Uruguay. *Nutr Cancer* 31, 132-137
9 Slattery M L, Benson J, Berry T D, Duncan D, Edwards S L, Caan B J & Potter J D (1997) Dietary sugar and colon cancer. *Cancer Epidemiol Biomarkers Prev* 6, 677-685
10 Heuson J C, Coune A & Heimann R (1967) Cell proliferation induced by insulin in organ culture of rat mammary carcinoma. *Exp Cell Res* 45, 351-360
11 Osborne C K, Bolan G, Monaco M E & Lippman M E (1976) Hormone responsive human breast cancer in long term tissue culture: effect of insulin. *Proc Natl Acad Sci (USA)* 73, 4536–4540
12 Van Der Burg, B., Rutteman G R, Blankenstein, M A, De Laat, S W & Van Zoelen E J J (1988) Mitogenic stimulation of human breast cancer cells in a growth factor-defined medium: synergistic action of insulin and estrogen. *J Cell Physiol* 134,101-108
13 Novosyadlyy R, Lann D E, Vijayakumar A, Rowzee A, Lazzarino D A, Fierz Y, Carboni J M, Gottardis M M, Pennisi P A, Molinolo A A, Kurshan N, Mejia W, Santopietro S, Yakar S, Wood T L & LeRoith D (2010) Insulin-mediated acceleration of breast cancer development and progression in a nonobese model of type 2 diabetes. *Cancer Res* 70, 741–751
14 Belfiore A & Malaguarnera R (2011) Insulin receptor and cancer. *Endocrine-related Cancer* 18, R125-R147
15 Milazzo G, Giorgino F, Damante G, Sung C, Stampfer M R, Vigneri R, Goldfine I D & Belfiore A (1992) Insulin receptor expression and function in human breast cancer cell lines. *Cancer Res* 52, 3924–3930
16 Kasuga M, Kahn C R, Hedo J A, Van Obberghen E & Yamada K M (1981) Insulin-induced receptor loss in cultured human lymphocytes is due to accelerated receptor degradation. *Proc Natl Acad Sci (USA)* 78, 6917–6921
17 Tozzo E & Desbuquois B (1992) Effects of STZ-induced diabetes and fasting on insulin receptor mRNA expression and insulin receptor gene transcription in rat liver. *Diabetes* 41, 1609–1616
18 Secchi L A, Griffin C A, Grady E F, Grunfeld C, Kalinyak J E & Schambelan M (1992) Tissue specific regulation of the insulin receptor mRNA levels in rats with STZ-induced diabetes mellitus. *Diabetes* 41, 1113–1118
19 Maassen J A, Krans H M J & Moller W (1987) The effect of insulin, serum and dexamethasone on mRNA levels for the insulin receptor in the human lymphoblastoid cell line IM9. *Biochim Biophys Acta* 930, 72–78
20 Rouiller D G, McKeon C, Taylor S I & Gorden P (1988) Hormonal regulation of insulin receptor gene expression: hydrocortisone and insulin act by different mechanisms. *J Biol Chem* 263, 13185–13190
21 Okabayashi Y, Maddux B A, McDonald A, Logsdon C D, Williams J A & Goldfine I D (1989) Mechanisms of insulin-induced insulin receptor down-regulation. Decrease of receptor biosynthesis and mRNA levels. *Diabetes* 38, 182–187
22 Hatada E N, McClain D A, Ullrich A & Olefsky J M (1989) Effects of growth and insulin treatment on the levels of insulin receptors and their mRNA in HepG2 cells. *J Biol Chem* 264, 6741–6747
23 Rohilla A M K, Anderson C, Wood W M & Berhanu P (1991) Insulin down regulates the steady-state level of its receptor's messenger ribonucleic acid. *Biochem Biophys Res Commun* 175, 520–526
24 Bortoli S, Amessou M, Collinet M, Desbuquois B & Lopez S (1997) Vanadate, But Not Insulin, Inhibits Insulin Receptor Gene Expression in Rat Hepatoma Cells. *Endocrinology* 138, 4821-4829
25 Giorgino F, Belfiore A, Milazzo G, Costantino A, Maddux B, Whittaker J, Goldfine ID & Vigneri R (1991) Overexpression of insulin receptors in fibroblast and ovary cells induces a ligand-mediated transformed phenotype. *Mol Endocrinol* 5, 452–459
26 Rose P P, Carroll J M, Carroll P A, DeFilippis V R, Lagunoff M, Moses A V, Roberts C T Jr & Fruh K (2007) The insulin receptor is essential for virus-induced tumorigenesis of Kaposi's sarcoma. *Oncogene* 26, 1995–2005
27 Mathieu M C, Clark G M, Allred D C, Goldfine I D & Vigneri R (1997) Insulin receptor expression and clinical outcome in node-negative breast cancer. *Proc Assoc Am Physicians* 109, 565–571
28 Law J H, Habibi G, Hu K, Masoudi H, Wang M Y, Stratford A L, Park E, Gee J M, Finlay P, Jones H E, Nicholson R I, Carboni J, Gottardis M, Pollak M & Dunn S E (2008) Phosphorylated insulin-like growth factor-i/insulin receptor is present in all breast cancer subtypes and is related to poor survival. *Cancer Res* 68, 10238–10246

29 Osborne C K, Monaco M E, Lippman M E & Kahn C R (1978) Correlation among insulin binding, degradation, and biological biological activity in human breast cancer cells in long-term tissue culture. *Cancer Res* 38, 94-102

30 Mountjoy K G, Holdaway I M & Finlay G J (1983) Insulin receptor regulation in cultured human tumor cells. *Cancer Res*. 43, 4537-4542

31 Loeper S & Ezzat S (2008) Acromegaly: re-thinking the cancer risk. *Rev Endocr Metab Disord* 9, 41–58

32 The Endogenous Hormones and Breast Cancer Collaborative Group, Key T J, Appleby P N, Reeves G K & Roddam A W (2010) Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol* 11, 530–542

33 Rinaldi S, Cleveland R, Norat T, Biessy C, Rohrmann S, Linseisen J, Boeing H, Pischon T, Panico S, Agnoli C, Palli D, Tumino R, Vineis P, Peeters P H, van Gils C H, Bueno-de-Mesquita B H, Vrieling A, Allen N E, Roddam A, Bingham S, Khaw K T, Manjer J, Borgquist S, Dumeaux V, Torhild Gram I, Lund E, Trichopoulou A, Makrygiannis G, Benetou V, Molina E, Donate Sua´ rez I, Barricarte Gurrea A, Gonzalez C A, Tormo M J, Altzibar J M, Olsen A, Tjonneland A, Grønbaek H, Overvad K, Clavel-Chapelon F, Boutron-Ruault M C, Morois S, Slimani N, Boffetta P, Jenab M, Riboli E & Kaaks R (2010) Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-analysis of prospective studies. *Int J Cancer* 126, 1702–1715

34 Huang Y F, Shen M R, Hsu K F, Cheng Y M & Chou C Y (2008) Clinical implications of insulin-like growth factor 1 system in early-stage cervical cancer. *Br J Cancer* 99, 1096–1102

35 de Ostrovich K K, Lambertz I, Colby J K, Tian J, Rundhaug J E, Johnston D, Conti C J, DiGiovanni J & Fuchs-Young R (2008) Paracrine overexpression of insulin-like growth factor-1 enhances mammary tumorigenesis in vivo. *Am J Pathol* 173, 824–834

36 Wu Y, Cui K, Miyoshi K, Hennighausen L, Green J E, Setser J, LeRoith D & Yakar S (2003) Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors. *Cancer Res* 63, 4384–4388

37 Wu Y, Yakar S, Zhao L, Hennighausen L & LeRoith D (2002) Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. *Cancer Res* 62, 1030–1035

38 Arnaldez F I & Helman L J (2012) Targeting the insulin growth factor receptor 1. *Hematol Oncol Clin North Am* 26, 527–542

39 Zhao R, DeCoteau J F, Geyer C R, Gao M, Cui H & Casson A G (2009) Loss of imprinting of the insulin-like growth factor II (IGF2) gene in esophageal normal and adenocarcinoma tissues. *Carcinogenesis* 30, 2117–2122

40 Guertin D A & Sabatini D M (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12, 9-22

41 Efeyan A & Sabatini D M (2010) mTOR and cancer: many loops in one pathway. *Curr Opin Cell Biol* 22, 169-176

42 Bhaskar P T & Hay N (2007) The two TORCs and Akt. *Dev Cell* 12, 487-502

43 Dancey J E (2002) Clinical development of mammalian target of rapamycin inhibitors. *Hematol Oncol Clin North Am* 16, 1101-1114

44 Zhou H & Huang S (2010) mTOR signaling in cancer cell motility and tumor metastasis. *Crit Rev Eukaryot Gene Expr* 20, 1-16

45 Kwon H K, Bae G U, Yoon J W, Kim Y K, Lee H Y, Lee H W & Han J W (2002) Constitutive activation of p70S6k in cancer cells. *Arch Pharm Res* 25, 685-690

46 Kenerson H L, Aicher L D, True L D & Yeung R S (2002) Activated mammalian target of rapamycin pathway in the pathogenesis of tuberous sclerosis complex renal tumors. *Cancer Res* 62, 5645-5650

47 Vogt P K (2001) PI 3-kinase, mTOR, protein synthesis and cancer. *Trends Mol Med* 7, 482-484

48 Gao N, Flynn D C, Zhang Z, , Zhong X-S , Walker V , Liu K J , Shi X & Jiang B-H (2004) G_1 cell cycle progression and the expression of G_1 cyclins are regulated by PI3K/AKT/mTOR/p70S6K1 signaling in human ovarian cancer cells. *Am J Physiol* 287, C281-C291

49 Pópulo H, Lopes J M & Soares P (2012) The mTOR Signalling Pathway in Human Cancer. *Int J Mol Sci* 13, 1886-1918

50 Alquarashi N, Gopalan V, Smith R A & Lam A K (2013) Clinical impacts of mammalian target of rapamycin expression in human colorectal cancers. *Hum Pathol* 44, 2089-2096

51 Kremer C L, Klein R R, Mendelson J, Browne W, Samadzedeh L K, Vanpatten K, Highstrom L, Pestano G A & Nagle R B (2006) Expression of mTOR signaling pathway markers in prostate cancer progression. *Prostate* 66, 1203-1212

52 Yu G, Wang J, Chen Y, Wang X, Pan J, Li G, Jia Z, Li Q, Yao J C & Xie K (2009) Overexpression of Phosphorylated Mammalian Target of Rapamycin Predicts Lymph Node Metastasis and Prognosis of Chinese Patients with Gastric Cancer. *Clin Cancer Res* 15, 1821-1829

53 No J H, Jeon Y T, Park I A, Kang D, Kim J W, Park N H, Kang S B & Song Y S **(**2009) Expression of mTOR protein and its clinical significance in endometrial cancer. *Med Sci Monit* 15, BR301-305

54 Courtney K D, Corcoran R B & Engelman J A (2010) The PI3K pathway as drug target in human cancer. *J Clin Oncol* 28, 1075-1083

55 Alvarez M, Roman E, Santos E S & Raez L E (2007) New targets for non-small-cell lung cancer therapy. *Expert Review of Anticancer Therapy* 7, 1423–1437

56 Dufour M, Dormond-Meuwly A, Demartines N & Dormond O (2011) Targeting the Mammalian Target of Rapamycin (mTOR) in Cancer Therapy: Lessons from Past and Future Perspectives. *Cancers* 3, 2478-2500

57 Burris H A 3rd (2013) Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway. *Cancer Chemother Pharmacol* 71, 829-842