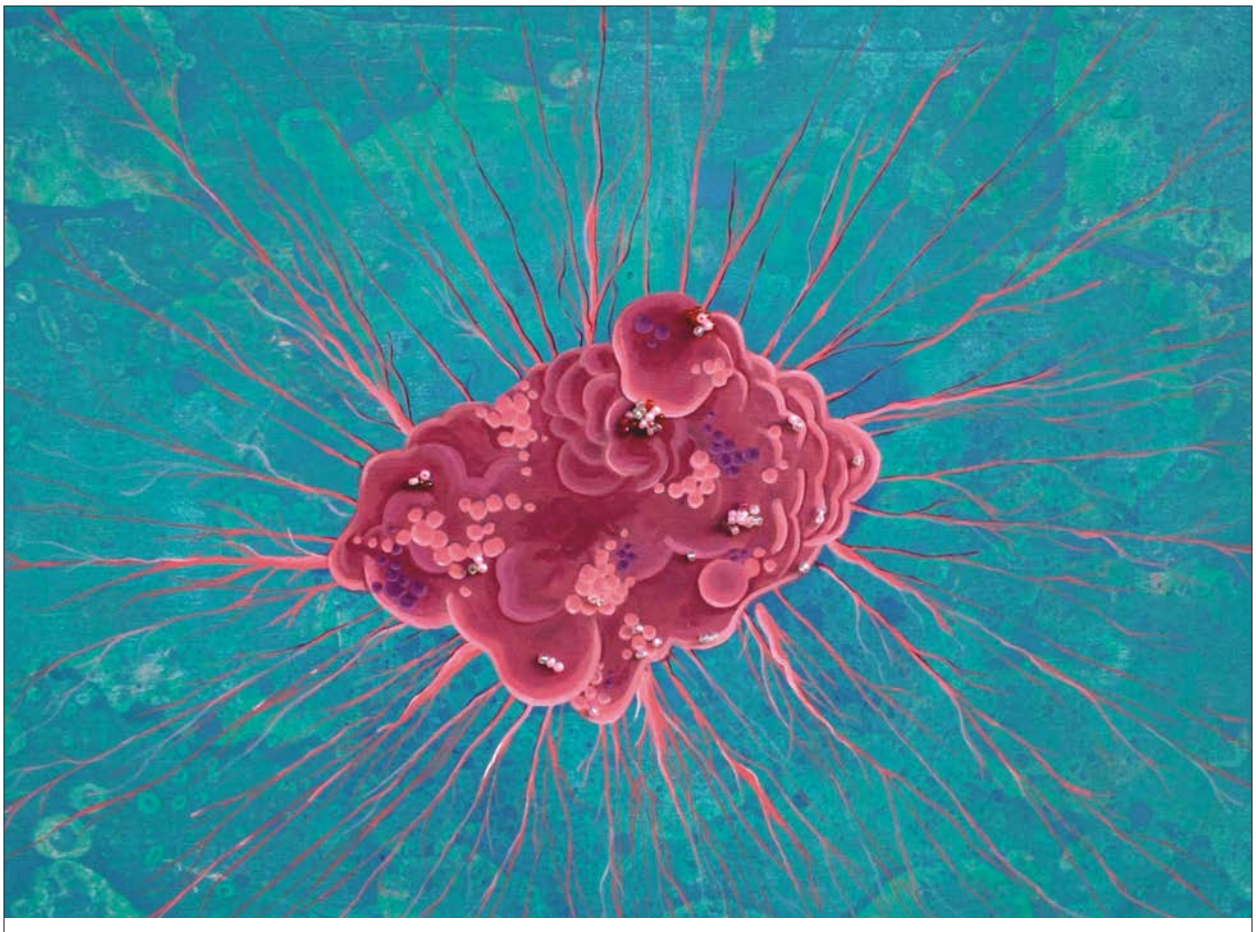

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Insulin like Growth Factor-1 Receptor: An Anticancer Target Waiting for Hit

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Abstract: The Insulin like Growth Factor-1 (IGF-1) receptors are members of the super family of Receptor Tyrosine Kinase (RTKs) implicated in human cancers due to amplification, overexpression or somatic mutation of the gene. The type-1 insulin like growth receptors (IGF-1R) is overexpressed by many tumors and mediates proliferation, motility and apoptosis protection. Tumor growth and metastasis can be blocked by agents that inhibit IGF-1R expression or function, suggesting that the IGF-1R is a promising treatment target. The strategies to block IGF-1R function employed anti-IGF-1R antibodies, small-molecule inhibitors of the IGF-1R tyrosine kinase, antisense oligodeoxynucleotides and antisense RNA, small inhibitory RNA, triple helix, dominant negative mutant and various compounds reducing ligand availability. Studies show that antisense IGF-1R expression in melanoma cells leads to enhanced radio sensitivity and impaired activation of ATM, required for DNA double strand break repair. Antisense and dominant negative strategies also enhance tumor cell chemosensitivity induced by tumor cells and killed *in vivo* by IGF-1R antisense. However, antisense agents cause only modest IGF-1R down regulation and can affect the insulin receptor. Specificity is an important issue for the development of both kinase inhibitors and molecular reagents. Using an array-based screen to identify accessible region of IGF-1R mRNA, are designed small interfering RNAs (siRNAs) that induce potent IGF-1R gene silencing without affecting the insulin receptor. These siRNAs block IGF-signaling, enhancing radio and chemosensitivity and show a genuine therapeutic potential. The clinical efficacy of IGF-1R targeting will be determined by key factors including the role of receptors in established tumors. The potency of inhibition achieved *in vivo* and the extent to which other signaling pathways compensate for IGF-1R loss.

Key words: Insulin like growth factors, cancer, insulin, antisense, IGF-IR inhibitors

Introduction

Tumor cells exhibit abnormally high levels of proliferation, which is promoted and controlled by a variety of growth factors. Among these, the Insulin-like Growth Factors (IGFs) play a major role in regulating cell proliferation and inhibiting apoptosis. IGFs are expressed ubiquitously and act as an autocrine/paracrine manner through binding to the IGF-I receptor (IGF-IR). The bioactivity of IGF in tissues is determined by both local and systemic factors. The local factors included the levels of the receptors that are expressed, various IGF binding protein (IGFBPs) and IGFBP protease. The systemic factors involved are mainly those that regulate the circulating levels of IGFs, such as Growth Hormone (GH) and various nutritional factors. IGF system is comprised of the IGF ligands (IGF-I and IGF-II), cell surface receptors that mediate the biological effects of the IGFs including the IGF-IR, the IGF-II receptor (IGF-IIR) and Insulin Receptor (IR), as well as a family of IGF-binding proteins (IGFBPs). IGFBPs affect the half-lives and bioavailability of the IGFs in the circulation, in

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extracellular fluids and may exert IGF-independent effect under certain conditions. This review will focus on the structure and function of the components of the IGF axis, their interactions and their role in tumerogenesis (Baxter, 2000; Clemmons, 2001; LeRoith *et al*., 1995).

IGF Ligands

Structure of IGF-I and IGF-II

The mature IGF-I and IGF-II peptides consist of B and A chain of insulin. Unlike insulin, the IGF peptides are not prototypically cleaved, but remain linked in the mature peptides by C domains analogous to the C peptide of insulin. Both IGF-I and IGF-II contain an additional short D domain that is not found in insulin. The IGF-I and IGF-II prohormones contain a C-terminal E peptide that is cleaved in the Golgi apparatus during secretion (Daughaday and Rotwein, 1989).

Expression of IGF-1 and IGF-II

The prenatal expression of the IGF-II gene is widespread in rodent and diminished dramatically after birth only with the choroids plexus and the leptomeninges persistently synthesizing IGF-II in adult animals. In contrast, murine expression of IGF-I is low during the prenatal period and increases significantly during puberty and adulthood, when hepatic productions become a major contributor to overall circulating IGF-1 levels. IGF-I exerts endocrine, paracrine and autocrine effects and is produced by numerous other adult organs, including kidney, lung and bone. The overall picture of IGF expression in rodent initially led to the concept of IGF-II as a fetal growth factor and IGF-I as an adult growth factor. However, this expression pattern is not observed in human; as both IGF-I and IGF-II are produced in multiple human tissues throughout life. Infact, the circulating levels of human IGF-II are consistently several fold higher than that of IGF-I, which is consistent with the concept that IGF-I and IGF-II have potentially divergent roles in human physiology (Daughaday and Rotwein, 1989).

IGF Receptors

IGF and Insulin Receptors

The IGF-I and IGF-II ligands interact with an array of cell surface receptors that may be present singly or in various combinations on target cells. Both IGF-I and IGF-II interact with the IGF-IR, a transmembrane tyrosine kinase that is structurally and functionally related to the IR. IGF-II can also bound to the IGF-II-R with high affinity. Cloning of the previously characterized cation-independent mannose-6-phosphate (M6P) receptor, which plays a role in endocytosis and intracellular trafficking of M6P-tagged proteins. This molecule is thought to function as a clearance receptor for IGF-II, thereby enhancing the extra cellular levels of IGF-II (Daughaday and Rotwein, 1989). Most, if not all, of the effects of IGF-I result from activation of the IGF-IR. IGF-I does not cross react with the IR, except at pharmacological doses, since IGF-I has a relative affinity almost two order of magnitude higher for the IGF-IR, as compared to the IR. Until recently, it was thought that IGF-II like IGF-I, bound at significant levels to the IGF-IR but not to the IR. Studies of knockout mice lacking various components of the IGF and insulin receptor systems suggested that IGF-II acted through the IR during the early stages of development, before IGF-IR gene expression was detectable. The molecular basis for the phenomenon was revealed when it was discovered that a splice variant of the IR displayed high affinity for IGF-II. The IR transcript is subject to alternative splicing of exon 11, which encodes a 12-aminoacid segment in the C-terminus of the extra cellular subunit. Previous studies had shown that the IR isoform encoded by the mRNA lacking the exon 11 sequence (IR-A) displayed a 2 fold higher affinity for insulin than the IR-B form, which includes exon 11. More recently, it has been reported that the IR-A isoform functions as a high affinity receptor for IGF-II and mediates predominantly

proliferative effects as compared to the principally metabolic effects elicited by insulin stimulation of the IR-B isoform (Rother and Accili, 2000). Thus, IGF-1 functions primarily by activating the IGF-IR, whereas IGF-II can act through either the IGF-IR or through the IR-A isoform.

Hybrid Receptors

The complexity of IGF signaling is increased by the formation of hybrid receptors that result from the dimerization of IGF-IR and IR hemireceptors. Each hybrid receptor consists of a single α and β subunits linked by disulfide bonds, which are formed in the Golgi apparatus of cells expressing both the IGF-IR and the IR. These could be due to the preferential formation of disulfide bonds between cysteine residue in IGF-IR and IR subunit themselves. Thus, in some circumstances, hybrid receptors may outnumber homoreceptor molecules at the cell surface. IGF-IR/IR hybrid receptors retain high affinity for IGF-I, but exhibit a dramatically decreased affinity for insulin. This is thought to reflect the ability of IGF-I to efficiently bind to either IGF-IR α. Whereas tight insulin binding requires interaction with both of the β subunit found in the IR. Thus the presences of a significant number of hybrid receptor may selective diminish cellular responsiveness to insulin, but not to IGF-I. Indeed, this has been proposed as a mechanism by which up-regulation of IGF-IR expression could cause insulin resistance in cells that express the IR. The effect of hybrid receptors are further complicated by the presence of the IR-A and IR-B isoforms and their different IGF-II binding characteristics. It is likely that hybrids formed between both IR-A/IGF-IR and IR-B/IGF-IR form, since most cells express both splice variants. It has been recently demonstrated that IGF-IR/IR-A hybrids bind IGF-I, IGF-II and insulin, whereas IGF-IR/IR-B hybrids bind IGF-I with high affinity, IGF-II with low affinity and do not bind insulin (Frasca *et al*., 1999). Therefore, the relative expression of the IGF-IR and IR genes and the degree of alternative splicing of exon 11 of the IR gene governs the ability of a given cells respond to IGF-I, IGF-II and insulin. It confirms potential receptor hybrid that may be involved in IGF signaling.

IGF-IR and IR Signal Transudation

IGF-IR is an evolutionary conserved, ubiquitous transmembrame tyrosine kinase, structurally similar to the Insulin Receptor (IR) (Pandini *et al*., 2002). IGF-IR is composed of two extracellular α subunits and two extracellular β subunits. The α subunit have ligands (IGF-I, IGF-II and insulin at supraphysiological doses) binding site, while β subunit contain three major domains; the juxtamembrane domain, tyrosine kinase domain and C-terminus. The tyrosine kinase domain share high (85%) homology with its counterpart in IR, while the C-terminus is only 40% homologous with the C-terminus of IR (Pandini *et al*., 2002). Binding of ligands to IGF-IR induces its autophosphorylation and tyrosine phosphorylation of IGF-IR substrate, especially the IR substrate 1 (IRS-1) and src and collagen-homology (SHC) protein. Tyrosine-phosphorylated IRS-1 and SHC bind different effector proteins (enzymes and/or adaptors) inducing multiple signaling cascades, among them several interconnecting pathways controlling cell survival and proliferation (Ullrich, 1986; Shepherd *et al*., 1998; White, 1994, 2002; Adams *et al*., 2000; Surmacz, 2000). The critical survival pathway activated by IGF-I stems from IRS-1. IRS-1 recruits and stimulates the PI-3 kinase (PI-3k), which then transmits signal to the serine/threonine kinase Akt. The activated Akt phosphorylates and block a variety of propoptotic proteins, including BAD, caspase-9, beta kinase. Furthermore, Akt induces the expression of antiapoptotic proteins, for example, Bcl-2 (Brazil *et al*., 2002; Hill and Hemmings, 2002; Nicholson and Anderson, 2002). Other mitogenic survival of IGF-IR pathways involves signal transducers and activators of transcription (STATs) that are phosphorylated and activated by IGF-I through JAK-1/2 and PI-3k/Akt pathway (Zong *et al*., 2000). In addition, IGF-IR can prevent cell death or induce proliferation via the SHC/Ras/ERK1/2 pathway (Zong *et al*., 1998; Peruzzi *et al*., 1999). While antiapoptotic and growth pathways of IGF-IR have been extensively studied, the signals

controlling non-mitogenic functions of IGF-IR, such as well substrate adhesion, migration, invasion, or intracellular interactions are not well understood. There is increasing evidence that IGF-IR pathways interconnect with interin and cadherin signaling system (Vuori and Ruoslahti, 1994). In some experimental model, IGF-IR has been shown to mediate metastasis, possibly through-enhanced migration (Doerr and Jones, 1996; Bartucci *et al*., 2001) reduced cell-cell adhesion (Mauro *et al*., 2003) and upregulation of Plasminogen Activator (PA) and matrix metalloproteinase (Long *et al*., 1998; Mira *et al*., 1999; Dunn *et al*., 1998; Zhang and Brodt, 2003).

Insulin like Growth Factor Binding Protein (IGFBP)

The biological activities of the IGF ligands are also modulated by a family of high affinity IGFBP-1 to 6 that are found in the circulation and in extra cellular fluids. IGFBP-3 is the predominant IGFBP in serum and the most circulating IGF-I and IGF-II are not found in free form, but as a ternary complex with IGFBP-3 and the acid-labile subunit (ALS), in 1:1:1 molar ratio. IGFBP-5 also form ternary complexes with IGFs and ALS. While IGFBPs-1 through 4 generally has similar affinity for IGF-I and IGF-II. IGFBP-5 and 6 bind IGF-II with 10 and 100-fold greater affinities respectively, than IGF-I. The IGFBPs do not bind with insulin (Guvakova and Surmacz, 1999; Mauro *et al*., 1999). The IGFBPs control IGF action by increasing the half lives of circulating IGFs, by controlling their availability for receptor binding and in the case of cell surface associated IGFBPs; by potentially influencing their direct interaction with receptors (Baxter, 2000). Thus ligand receptor interactions in the IGF system are subjected to complex regulation as a result of the levels of IGFBPs, their expression profile, the degree of cell surface association and the extent of proteolysis. A series of studies performed over the past several year has established that certain actions of the IGFBPs are IGF-independent. IGFBP-3 and IGFBP-5, in particular have been shown to exhibit effects on proliferation migration and sensitivity to apoptosis that are independent of their effects on IGF-signaling per se. Some of these IGF-independent effects are still modulated by IGF-binding to the particular IGFBP, so 'IGF-receptor-independent actions' may be a more accurate term for these novel functions. The cell surface and/or intracellular molecule that participate in these effects have not been well characterized, but exposure to exogenous recombinant IGFBP-3 and IGFBP-5 proteins has been shown to induce nuclear localization of these proteins. A better characterization of these IGF-receptor-independent actions of IGFBPs will provide an important new dimension to our understanding of the IGF signaling system in general (Reiss *et al*., 2001; Shaw, 2001).

Physiological and Pathophysiological aspects of IGF Action

The IGF system plays an important role in normal growth and development as well as in a variety of pathological situations, particularly tumerogenesis (Khandwala *et al*., 2000). IGF action is also important in the development of specific organ, such as in the nervous system, in which IGF-signaling regulate neuronal proliferation, apoptosis and cell survival. IGF action plays a critical role in the development and progression of human cancer. A growing body of epidemiological data suggested that high levels of circulating IGF-1 constitute the risk factor for the development of breast, prostate, colon and lung cancer outcomes. As a result of some experimental findings, intensive efforts is being directed towards investigating the utility of the IGF system as both a diagnostic marker and a therapeutic target in cancer therapy.

Role of IR Signaling Cascade in Cancer Cell Function

Regulation of IGF-IR gene expression is closely associated with the function of a variety of tumor suppressor genes and oncogenes. The p-53 tumor suppressor protein protects mammalian cells against

cancer. A large number of human cancer cells exhibit mutations within the p-53 gene that either impair its tumor suppressor function or provide it with oncogenic potential. Expression of wild-type p-53 inhibits IGF-IR gene expression, whereas mutant p-53 upregulates IGF-IR gene expression (Werner *et al*., 1996). Mdm-2 targets p53 for degradation: Mdm-2 mediated reduction of p-53 could thereby induce upregulation of the IGF-IR and increases the survival of cancer cells (Girnita *et al*., 2000). Expression of the IGF-IR is also regulated by the src tyrosine kinase, PKB/Akt serine-threonine kinase and the PTEN protein phosphates. Constitutively active Akt or src- activated Akt upregulates IGF-IR gene expression, whereas PTEN counteracts this β effect in pancreatic cancer cells and render the cell more invasive (Tanno *et al*., 2001). Neuronuclear factor (NF) -κβ is a transcription factor that can function in both cytokine signaling and in cell survival. NF-κβ mediates antiapoptotic effect of IGF-I in colon cancer cells (Garrouste *et al*., 2002), whereas it can mediate proapoptotic effects under other circumstances such as its role in the effects of tumor necrosis factor-α (Cheshire and Baldwin, 1997). Thus, the specific cellular response to NF-κβ depends on the original stimulus. Migration of epithelial colonic cells is dependent on IGF-IR induced alteration in integrins and cell adhesion complexes. While IGF-IR activation did not alter integrin expression levels, most of the integrin relocalized to the leading edge of migrating cells in response to IGF-I stimulation. Blocking integrin function with specific antibodies inhibited IGF-I induced migration. Furthermore, activation of the IGF-IR disrupts the E-cadherin/catenin complex, which associated with the cytoskeleton (Andre *et al*., 1999). Similarly, in MCF-7 breast cancer cells, the IGF-IR was showed to directly interact with the cells adhesion complex comprised of E-cadherin, β-catenin and p120 catenin. When IGF-IR antisense mRNA was expressed in MCF-7 cells, the cells exhibited a more malignant phenotype that was associated with a reduction in cell-cell adehision complex. This reduction was proposed to arise from a p120 catenin-induced decrease in E-cadherin and activation of Rac and Cad 42 activity (Pennisi *et al*., 2002). Certain tumor cells exhibit growth factor dependence in early, during the progression of tumorigenesis. During later stages, such cells may become growth factor, which independent for continued progression. For example, early stage melanoma cells have recently been shown to be exquisitely sensitive to IGF-I. At these early stages, IGF-I activates the MAP kinase pathway, which triggers proliferation and the PI kinase pathway, which promotes cell survival and stabilization of β-catenin. At the later stages of development i.e., in malignant melanoma cells, Erk 1 and Erk 2 were constitutively activated and β-catenin become more stabilized; IGF-I was unable to further activate these system (Satyamoorthy *et al*., 2001). Cross talk between receptors and their signaling pathway has been recently shown to play a critical role in various cellular responses to ligands. Such cross talk may occur between receptors within the same family, such as the Epidermal Growth Factor (EGF) and IGF-I receptor, both are tyrosine kinase receptors (Gilmore *et al*., 2002) or between different families such as nuclear steroid receptors and the IGF-IR (Dupont *et al*., 2000) or G-protein coupled receptors and the IGF-IR (Dalle *et al*., 2001). For example, the GBM(R) glioblastoma cell line is insensitive to AG1478, an anti-EGF therapeutic agent that acts as a specific EGF- receptor tyrosine kinase inhibitor. GBM (R) cells exhibited compensatory upregulation of IGF-IR levels in response to AG1478 treatment. This resulted in persistent signaling through the PI3 kinase pathway and was associated with an antiapoptotic and proinvasive phenotype. Both Akt1 and p70S6K appeared to play a role in this process (Chakravarti *et al*., 2002). In another example, the IGF-IR also protects mammary epithelial cells from apoptosis. Activation of this IGF-IR induces serine phosphorylation of BAD in this cell type, but this is mediated via transactivation of the IGF receptor, as this effect was blocked by ZD1839, a specific EGFR tyrosine kinase antagonist (Gilmore *et al*., 2002). Motility is an important process that plays a role in the spread of cancer cells. Activation of the IGF-IR and subsequent activation of the PI3 kinase pathway induces extension of lamellipodia in neuroblastoma cell lines (Meyer *et al*., 2001). Migration of melanoma cells is also stimulated by IGF-I. This effect is mediated by upregulation of interleukin-8 gene suppression via IGF-I induced activation of MAP

kinase and phosphorylation of c-Jan (Satyamoothy *et al*., 2002). Various strategies have been used to block the IGF-IR in order to prevent tumor cell growth and to increase apoptosis of malignant cells. Expression of a dominant- negative truncated IGF-IR in colon cancer cells reduces the level of vascular endothelial growth factor expression, impaired tumor progression in nude mice and increase tumor cell apoptosis (Reinmuth *et al*., 2002). Scotlandi *et al*. (2002a, b) overexpresses a dominant negative form of this IGF-IR with a mutated ATP-binding site in enhanced apoptosis, decreased tumorigenesis and increased sensitivity to chemotherapeutic agents. Other techniques that have been used to inactivate the IGF-IR include expression of truncated soluble receptors to prevent ligand-receptor interaction (Pietrzkowski *et al*., 1993) and expression of peptides that could interfere with these interaction (D'Ambrosio *et al*., 1996). Perhaps the most exciting potential therapeutic modalities will arise from the recent crystallographic studies of the tyrosine kinase domain of the IGF-IR (Faveylyukis *et al*., 2001; Munshi *et al*., 2002; Pautsch *et al*., 2001). The production of small molecule that can act as a specific antagonist for IGF-IR and it also inhibit its anti-apoptotic effects (Katia *et al*., 2005; De Meyts and Whittaker, 2002).

IGF-IR Structure-function Studies

The extensive mutational analysis of IGF-IR identified that the receptor domains required for initiation of specific function, that is, proliferation (measured as cell growth in monolayer), survival (usually measured as the ability of cell to survive under anchorage-independent conditions) and transformation (assessed as the ability to grow in soft agar or to form foci). The experiments by using different cell models unequivocally demonstrated the mutation in the ATP binding site of the IGF-IR tyrosine kinase domain produced 'dead' receptors, incapable of signal transmission. Mutations at other residues of the tyrosine kinase impair partially the IGF-IR. For instance, the substitutions of tyrosine (tyr1131, 1135 and 1136) into phenylalanine abrogate signal transformation and mitogenesis, but not survival signaling. Mutations in either Tyr1131 or Tyr1135 downregulate transformation without reducing cell growth. Tyr 950 in the IGF-IR juxtamembrane domain was found necessary for IRS and SHC binding and for induction of mitogenic and transforming activity, but the IGF-IR Tyr-950 mutant still transmitted in addition to the classic IRS-1 dependent PI-3k (Akt pathway). Other survival pathway(s) emanate from IGF-IR (Hongo *et al*., 1996; O'Connor *et al*., 2000; Romano *et al*., 1999). Deletion of the entire C-terminus at aa1229 totally abrogated transforming function, without inhibiting mitogenic and antiapoptotic ability (Surmacz *et al*., 1995). The 'transforming domain' was mapped between residues 1245 and 1310 with Tyr1251, Ser 1280-1283, His 1293 and Lys 1294 that are required for transformation. It is worth nothing that C-terminal deletions (at residue 1229 or 1245) appeared to amplify antiapoptotic effects and intrinsic inhibitor of IGF-IR survival signaling. The mutation in the C-terminus at Tyr 1250, 1251, His 1293 and Lys 1294 reduce survival, implying that these residues act as neutralizers of the C-terminus proapoptotic function (Hongo *et al*., 1996). The important practical implication of the above studies is that transformation by IGF-IR does not occur without activated IGF-I survival pathway. Thus targeting the survival function of IGF-IR, which should be the optimal approach to inhibit tumorigenicity, as evidenced by the mutational analysis, the best way to achieve this effects is to inactivate totally the IGF-IR tyrosine kinase.

Role of Circulating IGF in Cancer

Prostate Cancer

The potent mitogenic activity of IGF-I in cell culture made it an obvious candidate risk factor in cancer development, but it was not until 1998 that several prospective studies suggested that high circulating levels of IGF-I were associated with an increased risk of developing prostate cancer

(Cohen, 1998; Wolk *et al*., 1998). A significant amount of data had been accumulated suggest that the IGF system plays an important role in the prostate. Prostatic stromal cells and epithelial cells in primary culture secrete IGFBPs and stromal cells, which produce IGF-II and both stromal and epithelial cells express the IGF-I receptor, which are responsive to IGF-I with respect to proliferation (Cohen *et al*., 1994a-c). *In vivo*, it is likely that the prostate epithelial cells that are precursors to prostatic intraepithelial neoplasia and prostatic adenocarcinoma, respond to both locally produced IGF-II and circulating IGF-I through paracrine and endocrine mechanisms, respectively. Further support for the role of IGF-action in prostate growth has come from recent reports that systemic administration of IGF-I increases rat prostate growth (Torring *et al*., 1997), that modulate the ventral prostate weight by finasteride, is associated with altered levels of IGF-I receptors and IGFBPs gene expression (Huynh *et al*., 1998). The IGF-I deficient mice exhibit decreased prostate size and complexity of prostate structure (Ruan *et al*., 1999). The validity of the association between IGF-I levels and prostate cancer risk was questioned by subsequent cross sectional studies (Cutting *et al*., 1999; Djavan *et al*., 1999), in a prospective study, it was found that the IGF-I/PSA ratio was superior to IGF-I or PSA measurement alone for predicting prostate cancer risk. Finne *et al.* (2000), in a screening trial, did not find an association between serum IGF-I levels and prostate cancer risk, while Baffa *et al*. (2000) actually found that circulating IGF-I levels were lower in a group of patients undergoing radical prostatactomy as compared to age-matched controls. In prospective studies, however, Harman *et al.* (2000) and Stattin *et al.* (2000) found that IGF-I levels were associated with prostate cancer risk and this association was especially evident in younger men. While the conclusion of this extensive series of studies conducted over the last 4 years appears contradictory, there is, in fact, some consistency. Prospective studies consistently demonstrated an association between high circulating IGF-I levels and prostate cancer risk, while cross-sectional studies consistent have generated variable results. These data are consistent with the hypothesis that high serum IGF-I levels in younger men predict the occurrence of advanced prostate cancer. Years later, while IGF-I levels at the time of diagnosis are not especially informative. This hypothesis suggests that long–term exposure of prostate epithelial cells leads to high levels of serum-derived IGF-I, which increases the probability of initiating hyperplasia in the cellular precursors of prostatic intraepithelial neoplasia and subsequent prostate adenocarcinoma. Molecular corporation of the relationship between IGF-I levels and prostate carcinogenesis has now come from the analysis of transgenic mice with targeted expression of IGF-I in the basal prostatic epithelium. This dysregulated IGF-I biosynthesis regulated in the appearance of hyperplastic lesions resembling PIN by 6 months of age and prostatic adenocarcinomas or small cell carcinomas were eventually seen in 50% of the transgenics. Specifically, desregulated expression of IGF-I and constitutive activation of IGF-I receptor in basal epithelial cells resulted in tumor progression similar to that seen in human disease. These studies also provide additional evidence for the prostate basal epithelial cell as a precursor to prostate cancer.

Breast and Other Cancer

Hankinson *et al.* (1998) reported that premenopausal, but not post-menopausal women in the highest fertile of serum IGF-I levels had a significantly increased risk of developing breast cancer. These findings have been generally supported by most (Vadgama *et al*., 1999; Jernstrom and Barret-Connor, 1999) but not all subsequent studies. Racial factors may play a role in the IGF-I breast cancer association, in the Agar-Collins *et al.* (2000) found that high serum IGF-I levels were strongly associated with breast cancer risk in post menopausal African-American women. With respect to the colorectal cancer, Ma *et al.* (1999) and Palmqvist *et al.* (2002) have reported positive association between serum IGF-I and colorectal cancer risk in US, Greek and Swedish cohorts, while Probst-Hansch *et al.* (2001) found an association between IGF-I or IGFBP-3 levels and colorectal cancer risk in a Chinese cohorts. The role of IGF-II is also unclear, being positively associated in the

Greek and Chinese studies, but not in the US cohorts. Yu *et al.* (1999) reported a positive association between high IGF-I and low IGFBP-3 levels (but not IGF-II) and lung cancer risk. Lukanova *et al.* (2001), however found no correlation between serum IGF-I or IGFBPs in a large female cohort. Collectively, these studies continue to suggest a role for IGF-I at a risk factor for breast, colorectal and lung cancer, but its utility as a pragmatic marker is potentially limited by ethnic and (for colorectal and lung) gender factors.

In breast cancer cells, estrogens enhance the mitogenic effect of IGF-I, induced expression of IGF-I and stimulate production of IGF-IR (Herbert and Thomas, 2000). Estrogens also repress synthesis of some IGFBPs in breast tissue. In breast cancer cells, estrogens decrease the expression of IGF-IIR and increase the level of IGFBP proteases (Clarke *et al.*, 1997). The interaction between estrogens and IGF is reciprocal. IGF-I enhances expression of estrogen receptor (ER) in breast cancer cells and ER levels in breast tissue are associated with the levels of some IGFBPs (Mathieu *et al.*, 1991).

Role of the IGF-IR in Human Cancer

Numerous studies performed over the last 20 year have suggested that transformed cells express the IGF-IR at higher levels than normal cells. However, the molecular mechanism which IGF-IR gene increases expression in tumors remains largely unidentified. Amplification of the IGF-IR locus at band 15q26 has been reported in a small number of breast cancer and melanoma cases (Almeida *et al.*, 2001). During tumorigenesis, overexpression of the IGF-IR is presumed to increases the cellular responsiveness to the IGFs, in terms of proliferation and inhibition of apoptosis. This picture is probably most accurate with respect to the pediatric tumors associated with chromosomal translocation such as Wilm's tumor and rhabdomyosarcoma. However, the role of the IGF-IR in the progression of epithelial tumors that are most prevalent in adults is likely to be more complex (De Pinho, 2000). It has been suggested that the IGF-IR itself can function as an oncogene, based upon the phenotype of fibroblasts overexpressing the IGF-IR (Kaleko *et al.*, 1990). However, the relevance of this system to human cancer in general is unclear. Other studies have used IGF-IR overexpression in fibroblasts to show that the IGF-IR can modulate radiosensitivity (Turner *et al.*, 1997). Nevertheless, it should be noted that a recent report demonstrated that inhibition of IGF-IR activity by a selective kinase inhibitor in MCF-7 breast cancer cells increases radiosensitivity (Wen *et al.*, 2001). Many studies describing overexpression of the IGF-IR in breast, prostate and other tumor cells have been largely based on analysis of tissue homogenates or established cancer cell lines. Unfortunately, there are no appropriate normal controls for these samples that can be used for comparison. This apparent IGF-IR content of tissue homogenates, in particular, can be affected by contamination with stroma, which would dilute the IGF-IR content in normal epithelium or small tumors. More focused studies of IGF-IR expression in breast and prostate that employed immunohisochemistry or matched cell lines corresponding to normal epithelium and early stage tumors both express abundant levels of the IGF-IR and that IGF-IR expression is significantly reduced in advanced, metastatic cancer (Tennant *et al.*, 1996; Happerfield *et al.*, 1999; Chott *et al.*, 1999; Schnarr *et al.*, 2002; Damon *et al.*, 2001). A recent report Hellawell *et al.* (2002) challenged this view, reporting that IGF-IR expression was decreased in certain metastatic prostatic cancer. Sample, as compared with benign or carcinoma tissue, but that IGF-IR expression was increased in the majority of samples studied (eight out of 12). However, it should be noted that IGF-IR immunostaining with a single β-subunit antibody was diffusely cytoplasmic in most samples, in contrast to the expected membrane localization reported by Chott *et al.* (l999) who used two different subunit antibodies. Thus, the levels of IGF-IR expression in the progression of prostate cancer have not been clearly established. Activation of the IGF-IR present in normal epithelium in response to elevated levels of circulating IGF-I may underlie the epidemiological data described above. In contrast, the subsequent decrease in IGF-IR (if this is an attempt by established cancers cells to counteract the potential differentiating effects of IGF-I at the

sites of metastasis. Alternatively, decreased expression of the IGF-IR may protect tumor cells from a novel, non-apoptotic form of programmed cell death that has been recently described as being triggered by the unliganded IGF-IR (Sperandio *et al*., 2002).

The IGF Receptor as Anticancer Treatment Target

Two factors underpin the concept of the IGF-IR as anticancer treatment target, relating to the function of the IGF-IR and its pattern of expression. The IGF-IR is expressed on the surface of most normal cells, but it is frequently overexpressed by tumors, including melanoma and cancers of the colon, pancreas and prostate (Hellawell *et al*., 2002; Bohula *et al*., 2003).

IGF-IR Inhibitors

IGF signaling can be inhibited by blocking either the expression or function of the IGF-IR.

Inhibitors of IGF-IR Function

Small Molecule Kinase Inhibitors

Chemical inhibitors have many advantages; they can be designed for solubility and stability and they can often be administered orally with high bioavailability (Dreves *et al*., 2003). Specificity is a major design hurdle for development of IGF-IR inhibitors, given the high degree of homology with the insulin receptor. Recent structural studies reveal regions of divergence within the IGF-IR and insulin receptor kinase domains, suggesting that it may be possible to design specific IGF-IR inhibitors (Garcia-Echeverria *et al*., 2003; De Meyts and Whittaker, 2002). High throughput technology combined with computer modeling is currently used to identify low molecular weight compounds blocking the IGF-IR tyrosine kinase. The first described IGF-IR inhibitors, tyrphostin AG538 and I-OmeAG, were modeled on the IR tyrosine kinase. The compounds inactivated the IGF-IR tyrosine kinase by blocking the substrate-binding site; however, cross reactivity with the IR tyrosine kinase was reported (Blum *et al*., 2000). Recent advances in the characterization of the three dimensional structures of IGF-IR and IR greatly facilitate the design of specific IGF-IR inhibitors I (De Meyts and Whittaker, 2002). Most importantly, crystallographic studies revealed conformational differences in the phosphorylate forms of IGF-IR and IR kinases, the factors allowing the development of selective therapeutics (Faveylyukis *et al*., 2001; Pautsch *et al*., 2001). Several new compounds with enhanced specificity towards IGF-IR and low cross reactivity with IR entered into preclinical studies. The examples include derivatives of pyrimidine and podophyllotoxin, disclosed in patent application WO 02/092599 and WO 02/102804, respectively, specific small inhibitors of IGF-IR are likely candidates to become anti-IGF-IR drugs. The positive experience with similar therapeutics, especially the possibility of oral delivery and low toxicity, makes this approach especially attractive.

The therapeutic potential of a novel kinase inhibitor of IGF-IR, NVP-AEW541, in Ewing's sarcoma, osteosarcoma and rhabdomyosarcoma, the three most frequent solid tumors in children and adolescents. NVP-AEW541 may be combined with vincristine, actinomycin D and ifosfamide, three major drugs in the treatment of sarcomas.

Blocking Antibodies

Monoclonal antibodies to the IGF-IR have been shown to inhibit the growth of a range of tumors *in vivo*. Efficacy can be limited by poor penetration and antimouse immune responses, although these problems may be avoided by use of single chain and for humanized antibodies (Ludwig *et al*., 2003). The initial approach to inhibit IGF-IR signaling was based on the use of IGF-IR blocking antibodies. The mouse Mab alpha-1R3 raised against the alpha domain of IGF-IR (Jacobs *et al*., 1986) inhibited

IGF-IR activation and IGF-IR-dependent mitogenicity in several cell types *in vitro*, including breast carcinoma (Artega *et al*., 1989; Artega, 1992), rhabdomyosarcoma (Kalebic *et al*., 1994), NSCLC (Zia *et al*., 1996) and Ewing's sarcoma (Scotlandi *et al*., 1998). However, in some cases alpha-1R-3 was ineffective in blocking IGF-1 sensitive tumors in animal models (Artega, 1992). Furthermore, it has been reported that alpha-1R-3 may exhibit agonistic abilities towards IGF-IR (De Leon *et al*., 1992; Kato *et al*., 1993). The mouse anti-IGF-IR Mab 391 inhibited IGF-IR autophosphorylation and signaling to Akt in several human cancer cell lines. Chronic treatment with Mab 391 resulted in down regulation of receptor through lysosome-dependent pathways (Hailey *et al*., 2002). Several other mouse anti-IGF-IR Mab were described (Li *et al*., 1993, 2000). One of them Mab 1H7, which blocks IGF-IR/IGF-I binding and IGF-IR dependent DNA synthesis was used to engineer a single chain humanized anti-IGF-IR sc Fv-Fc Ab and contains the Fc domain of human IGF-1 fused to the Fv region of 1H7 (Li *et al*., 2000). Treatment of MCF-7 breast cancer cells with Sc Fv-Fc for 2-24 h downregulated the levels of IGF-IR through the lysosomal endocytic pathways, rendering the cells refractory to IGF-I stimulation (Sachdev *et al*., 2003). Importantly, down regulation of IGF-IR by scFv-Fc occurred also in MCF-7 xenografts and was paralleled by reduced tumor growth (Sachdev *et al*., 2003). These are similar humanized Mabs will likely become a model for future drug development. Once their specificity towards IGF-IR and lack of IR-cross reactivity is demonstrated *in vivo*.

An antagonistic monoclonal antibody, designated EM164, inhibits the proliferation and survival functions of the IGF receptor in cancer cells. EM164 was initially selected by a rapid cell-based screen of hybridoma supernatants to identify antibodies that bind to IGF-IR but not to the homologous insulin receptor and that show maximal inhibition of IGF-I-stimulated autophosphorylation of IGF-IR. However, the inhibition of MCF-7 cell growth *in vitro* by EM164 can be attributed principally to a cytostatic effect, where EM164 treatment causes cells to accumulate in the G0-G1 state of the cell cycle (Erin *et al*., 2003).

Dominant Negative Protein

IGF-IR dominant negatives have been constructed as protein transacted within the β-subunit, capable of forming inactive heterodimers of mutant and wild type receptors unable to transduce downstream signals. Soluble IGF-IR dominant negative lack the transmembrame region and compete with wild type receptors for ligand binding. This strategy has been shown to suppress IGF-IR function, resulting in inhibition of growth and tumorigenicity (Dreves *et al*., 2003).

IGF-I Mimetic Peptide

A series of small IGF-I peptide analogues was designed by molecular modeling of the IGF-I protein (Pietrzkowski *et al*., 1992, 1993) to compete with IGF-IR ligands. The synthetic peptides were modeled on domains of IGF-I, as these domains contain the least similarity between IGF-I and insulin. One of the peptides JB-1 (modeled on the domain) effectively inhibit IGF-I dependent IGF-IR autophosphorylation and proliferation in several tumor cell lines. The analogues used at nano or micro molar concentrations exhibited good specificity for IGF-IR and low toxicity for cells in cell culture (Pietrzkowski *et al*., 1992, 1993) compounds against experimental tumors *in vivo* has never been assessed.

Inhibitors of IGF-IR Expression

Because of difficulties in designing specific small molecule IGF-IR kinase inhibitors other molecular approach have used to block IGF-IR expression. Antisense is the best characterized of these, but this is now being surprised by the recent demonstration that profound gene silencing can be induced in mammalian cells by small interfering RNAs (Elbashir *et al*., 2001).

Antisense nucleotides, Antisense RNA, siRNA, Triple helix

A variety of experiments employing antisense oligodeoxynucleotides (ODNs), antisense RNA and small interfering RNA (siRNA) demonstrated that IGF-IR-dependent tumorigenicity can be decreased or eliminated by blocking IGF-IR mRNA, thus inhibiting IGF-IR protein synthesis. Most of the reported anti-IGF-IR ODNs contained sequences complementary to the IGF-IR translation initiation site. The association of these reagents with IGF-IR mRNA produced heteroduplex that was cleaved by RNase. Multiple studies documented that anti IGF-IR ODNs (regular or phosphorotioate chemistry) at nanomolar concentrations decreased IGF-IR expression reduced cell proliferation and rodent cancer cell type in cell grown in culture (Pietrzkowski *et al*., 1993; Dreves *et al*., 2003; Resnicoff *et al*., 1994, 1995a, b; Muller *et al*., 1998; Coppola *et al*., 1999; Macauley *et al*., 2001; Pavelic *et al*., 2002). Furthermore, in some instances, treatment with ODNs in induced massive apoptosis and tumor regression in animal models was reported (Resnicoff *et al*., 1995a, b). However the reduction of IGF-IR expression was often incomplete even with high concentrations (100-500 nM) of target sequence (Macauley *et al*., 2001). Moreover, interactions of anti-IGF-IR ODNs with IR synthesis were reported (Bohula *et al*., 2003). To address these problem, Bohula *et al*. (2003) used scanning oligonucleotide array to probe the secondary structure of IGF-IR mRNA in order to identify target sequences that are accessible for ODNs and do not appear in IR mRNA. This strategy enabled selection of specific ODNs that effectively and selectively downregulated IGF-IR in human cancer cell lines. Furthermore, the accessible sequence were suitable target for anti-IGF-IR siRNAs. Indeed, some of the designed siRNAs were able to silence paralleled by repression of IGF-IR signaling (Bohula *et al*., 2003). In addition to ODNs and siRNA, different antisense IGF-IR RNA vectors containing fragments of IGF-IR cDNA cloned in 3'5' orientation were generated to inhibit IGF-IR expression. The vectors (plasmids or viruses) produced antisense RNA that hybridized with complementary sequences in IGF-IR mRNA, blocking IGF-IR synthesis. For instance, an antisense IGF-IR RNA against the first 309 bases of IGF-IR mRNA, delivered to cells by transfection or adenoviral infection, reduced IGF-IR expression. IGF-I dependent proliferation and survival in a number of human and rodent cell models, including endometrial cancer (Nakamura *et al*., 2000), Ewing's sarcoma (Scotlandi *et al*., 2002a, b) and rat glioblastoma (Resnicoff *et al*., 1994). The expression of this IGF-IR antisense efficiently inhibit tumorigenicity of cells grown as explants in experimental animals, most probably by induction of massive apoptosis (Resnicoff *et al*., 1994, 1995b; Scotlandi *et al*., 2002a, b). The same antisense construct inhibit metastasis of murine lung carcinoma cells (Brodt *et al*., 2000). Another antisense IGF-IR plasmid containing ~300bp DNA complementary to the region surrounding the IGF-IR translation initiation site was used to inhibit IGF-IR expression and function in breast cancer cells (Neuenschwander *et al*., 1995). In many cases, the induction of cell death with antisense IGF-IR strategies was much more pronounced *in vivo* (animal models) than *in vitro* (monolayer tissues culture or soft agar), suggesting that *in vivo* tests may be superior in screening for anti-IGF-IR compounds (Resnicoff *et al*., 1994, 1995b; Scotlandi *et al*., 2002a, b). Oligonucleotide-directed triple helix formation is an approach to block transcription of specific genes by inhibiting the passage of RNA polymerase along with target DNA. The third effector strands (oligopyrimidine) contains oligopurine and/or oligopyrimidine sequenced in target DNA. The triple helix strategy has been reported to be effective in down regulation of IGF-IR. Specifically, a plasmid encoding the homopurine RNA sequences designed to form a triplex with a homopurine and homopyridine sequence present in 3' to the termination codon of the IGF-IR gene suppressed IGF-IR transcription in rat (6 glioblastoma cells). The triple helix reagent induced dramatic reduction of IGF-IR transcripts and IGF-IR expression can inhibit tumor formation in nude mice (Rininsland *et al*., 1997). Interestingly, in some case of rat C6 glioblastoma and some other cellular models, down regulation of IGF-IR by antisense approaches were associated with the induction of an immune host response leading to elimination of

untreated established tumors (Resnicoff *et al*., 1994). This peculiar effects perhaps related to the induction of immune response by the presence of apoptotic cells (Trojan *et al*., 2000) was further explored in pilot studies involving patients with astrocytomas treated with autologous glioma cells exposed to anti-IGF-IR ODNs (Andrews *et al*., 2001).

Modulators of IGF-IR Internalization and Recycling

Following ligand binding, the IGF-IR ligand complex is internalized and the ligand is degraded by endosomal proteinase and the receptor is returned to the membrane, one way to reduce IGF-I effects is to block IGF-IR re-expression on the cell surface. Recent studies suggested that IGF-IR trafficking could be substantially blocked by the inhibition of IGF-I degrading enzymes for example cathepsin. The cathepsin inhibitors, E-64 and CA074 methyl ester, reduced IGF-IR expression on the cell surface and impaired several IGF-I dependent effects, including DNA synthesis and synthesis of matrix metalloproteinase in human breast cancer and murine lung carcinoma cells (Brodt *et al*., 2000).

Perspective for Anti-IGF-IR Pharmaceuticals

IGF-IR is a promising target in cancer therapy because (1) IGF-IR expression is easily measurable by conventional techniques. (2) Tumor cells may be more sensitive to targeting IGF-IR than normal cells and (3) IGF-IR is often required for the tumorigenic effects of other oncogenic agents. Thus targeting IGF-IR can be combined with other therapies. Unlike with HER2 and EGFR, the development of anti-IGF-IR pharmaceutical is still in early discovery phases. Similarly to HER2 and EGFR, however, the most advanced strategies are those involving small inhibitors of the IGF-IR tyrosine kinase and anti-IGF-IR antibodies. Other approaches such as siRNA, antisense and triple helix strategies are also promising, but they will require optimization of specificity *in vitro* and efficient and safe delivery system.

Summary

In summary, the IGF signaling system plays a central role in many aspects of tumorigenesis. A better understanding of this complex system will facilitate the development of novel approaches to diagnose and treat various human cancers.

References

Adams, T.E., V.C. Epa, T.P. Garrett and C.W. Ward, 2000. Structure and function of the type-1 insulin-like growth factor receptor. Cell Mol. Life Sci., 57: 1052-1093.

Agrus-collins, T., L.L. Adams-Camppbell, K.S. Kim and K.J. Cullen, 2000. Insulin-like growth factor-I and breast cancer risk in postmenopausal African-American women. Cancer Detect. Prev., 24: 199-206.

Almeida, A., M. Muleris, B. Dutrillaux and B. Malfoy, 2001. The insulin-like growth factor-I, IGF-binding protein-1,2,3 and lung cancer risk in women. Int. J. Cancer, 92: 888-892.

Andre, F., J. Rigot, J. Thimonier, C. Montixi, F. Parat and G. Pommier *et al*., 1999. Integrins and E-cadherin cooperate with IGF-I to induce migration of epithelial colonic cells. Int. J. Cancer, 83: 497-405.

Andrews, D.W., M. Resnicoff, A.E. Flanders, L. Kenyon, M. Curtis and G. Merli *et al*., 2001. Results of a pilot study involving the use of an antisense oligodeoxynucleotides directed against the insulin-like growth factor type-I receptor in malignant astrocytomas. J. Clin. Oncol., 19: 2189-2200.

Artega, C.L., L.J. Kitten, E.B. Coronado, S. Jacobs, F.C. Kull Jr. and D.C. Allred *et al*., 1989. Blockade of the type-I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J. Clin. Invest., 84: 1418-1423.

Artega, C.L., 1992. Interference of the IGF system as a strategy to inhibit breast cancer growth. Breast Cancer Res. Treat., 22: 101-116.

Baffa, R., K. Reiss, E.A. El-Gabry, J. Sedor, M.L. Moy and D. Shupp-Byme *et al*., 2000. Low serum insulin-like growth factor-I (IGF-I): A significant association with prostate cancer. Tech. Urol., 6: 236-239.

Bartucci, M., C. Morelli, L. Mauro, S. Ando and E. Surmacz, 2001. Differential insulin-like growth factor-I receptor signaling and function in Estrogen Receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells. Cancer Res., 61: 6747-6754.

Baxter, R.C., 2000. Insulin like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. Am. J. Physiol. Endocrinol. Metab., 278: E967-E76.

Blum, G., A. Gazit and A. Levitzki, 2000. Substrate competitive inhibitor of IGF-1 receptor kinase. Biochemistry, 39: 15795-15812.

Bohula, E.A., M.P. Playford and V. Macaulay, 2003. Targeting the type I insulin-like growth factor receptor as anticancer treatment. Anticancer Drugs, 14: 669-682.

Brazil, D.P., J. Park and B.A. Hemmings, 2002. PKB binding proteins: Getting in on the Akt. Cell, 111: 293-303.

Brodt, P., A. Samani and R. Navab, 2000. Inhibition of the type-I insulin-like growth factor receptor expression and signaling: Novel strategies for antimetabolic therapy. Biochem. Pharmacol., 60: 1101-1107.

Chakravarti, A., J.S. Loeffler and N.J. Dyson, 2002. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res., 62: 200-207.

Cheshire, J.L. and A.S. Baldwin Jr., 1997. Synergistic activation of NF-kappa B by tumor necrosis factor alpha and gamma interferon via enhanced Iκ B α degradation and *de novo* Iκ, Bβ degradation. Mol. Cell Biol., 17: 6746-6754.

Chott, A., Z. Sun, D. Morganstern, J. Pan, T. Li and M. Susani *et al*., 1999. Tyrosine kinases expressed *in vivo* by human prostate cancer bone marrow metastases and loss of type 1 insulin like growth factor receptor. Am. J. Pathol., 155: 1271-1279.

Clarke, R.B., A. Howell and E. Anderson, 1997. Type I insulin-like growth factor receptor gene expression in normal human breast tissue treated with oestrogen and progesterone. Br. J. Cancer, 75: 251-257.

Clemmons, D.C., 2001. Use of mutagenesis to probe IGF-binding protein structure/function relationships. Endocr. Rev., 22: 800-817.

Cohen, P., D.M. Peehl and R.G. Rosenfeld, 1994a. The IGF axis in the prostate. Horm. Metab. Res., 26: 81-84.

Cohen, P., D.M. Peehl, B. Baker, F. Liu, R.L. Hintz and R.G. Rosenfeld, 1994b. Insulin-like growth factor axis abnormalities in prostatic stromal cells from patients with benign prostatic hyperplasia. J. Clin. Endocrinol. Metab., 79: 1410-1415.

Cohen, P., D.M. Peehl, H.C. Graves and R.G. Rosenfeld, 1994c. Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. J. Endocrinol., 142: 407-415.

Cohen, P., 1998. Serum insulin-like growth factor-I levels and prostate cancer risk-interpreting the evidence. J. Natl. Cancer Inst., 90: 769-783.

Coppola, D., B. Saunders, L. Fu, W. Mao and S.V. Nicosia, 1999. The insulin-like growth factor-1 receptor induces transformation and tumorigenicity of ovarian mesothelial cells and down regulates their Fas-receptor expression. Cancer Res., 59: 3264-3270.

Cutting, C.W., C. Hunt, J.A. Nisbet, J.M. Bland, A.G. Dalgleish and R.S. Kirby, 1999. Serum insulin-like growth factor-I is not a useful marker of prostate cancer. BJU Int., 83: 996-999.

Dalle, S., W. Ricketts, T. Imamura, P. Vollenweider and J.M. Olefsky, 2001. Insulin and insulin -like growth factor I receptors utilize different G protein signaling components. J. Biol. Chem., 276: 15688-15695.

D'Ambrosio, C., A. Ferber, M. Resnicoff and R. Baserga, 1996. A soluble insulin like growth factor I receptor that induces apoptosis of tumor cells *in vivo* and inhibit tumorigenesis, Cancer Res., 56: 4013-4020.

Damon, S.E., S.R. Plymate, J.M. Carroll, C.C. Springer, C. Dechsukhum and J.L. Ware *et al.*, 2001. Transcriptional regulation of insulin-like growth factor-I receptor gene expression in prostate cancer cells. Endocrinology, 142: 21-27.

Daughaday, W.H. and P. Rotwein, 1989. Insulin like growth factor I and II: peptide, messenger ribonucleic acid and gene structure, serum and tissue concentrations. Endocr. Rev., 10: 68-91.

De Leon, D.D., D.M. Wilson, M. Powers and R.G. Rosenfeld, 1992. Effects of insulin-like growth factors (IGFs) and IGF receptor antibodies on the proliferation of human breast cancer cells. Growth Factors, 6: 327-336.

De Meyts, P and J. Whittaker, 2002. Structural biology of insulin and IGF-I receptors: Implication of drug design. Natl. Rev. Drug Discov., 1: 769-783.

De Pinho, R.A., 2000. The age of Cancer. Nature, 408: 248-254.

Djavan, B., B. Bursa, C. Seitz, G. Soeregi, M. Remzi, A. Basharkhas and Wolfram *et al.*, 1999. Insulin-like growth factor-1 (IGF-1), IGF-1 density and IGF-1/PSA ratio for prostate cancer detection. Urology, 54: 603-606.

Doerr, M. and J. Jones, 1996. The role of integrins and extracellular matrix protein in the insulin-like growth factor-I stimulated chemotaxis of human breast cancer cells. J. Biol. Chem., 271: 2443-2447.

Dreves, J., M. Medinger, C. Schmidt-Gersbach, R. Weber and C. Unger, 2003. Receptor tyrosine kinases: The main target for new anticancer therapy. Curr. Drug Targets, 4: 1134-1121.

Dunn, S.E., M. Ehrlich, N.J. Sharp, K. Reiss, G. Solomon and R. Hawkins *et al.*, 1998. A dominant negative mutant of the insulin-like growth factor-I receptor inhibits the adhesion, invasion and metastasis of breast cancer. Cancer Res., 58: 3353-3361.

Dupont, J., M. Karas and D. LeRoith, 2000. The potentiation of estrogen on insulin-like growth factor I action in MCF-7 human breast cancer cells includes cell cycle components. J. Biol. Chem., 275: 35893-35901.

Elbashir, S.M., J. Harborth, W. Lendeckel, A. Yalcin, K. Weber and T. Tuschl, 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature, 411: 494-498.

Erin, K.M., L.M. Jennifer, E.D. Nancy, M.G. Lisa, M.C. Katherine, M.Z. Xiao, A.B. Walter, C. Thomas and S. Rajeeva, 2003. An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. Cancer Res., 63: 5073-5083.

Faveylyukis, S., J.H. Till, S.R. Hubbard and W.T. Miller, 2001. Structure and autoregulation of the insulin-like growth factor-I receptor kinase. Natl. Struct. Biol., 8: 1058-1069.

Finne, P., A. Auvinen, H. Koistinen, W.M. Zhang, L. Maattannen and S. Ranniko *et al.*, 2000. Insulin-like growth factor-I is not a useful marker of prostate cancer in men with elevated levels of prostate-specific antigen. J. Clin. Endocrinol. Metab., 85: 2744-2747.

Frasca, F., G. Pandini, P. Scalia, L. Sciacca, R. Mineo and A. Costantino *et al.*, 1999. Insulin receptor isoforms A: A newly recognized high affinity insulin like growth factor II receptor in fetal and cancer cells. Mol. Cell. Biol., 19: 3278-3288.

Garcia-Echeverria, C., J. Brueggen and H.G. Caparo *et al*., 2003. Characterization of potent and selective kinase inhibitors of IGF-IR. Proc. Am. Assoc. Cancer Res., 44: 1008-1015.

Garrouste, F., M. Remacle-Bonnet, C. Fauriat, J. Marvaldi, J. Luis and G. Pommier, 2002. Prevention of cytokine-induced apoptosis by insulin-like growth factor-I is independent of cell adhesion molecules in HT29-D4 colon carcinoma cell- evidence for a NF-kappa B-dependent survival mechanism. Cell Death Differ., 9: 768-779.

Gilmore, A.P., A.J. Valentijn, P. Wang, A.M. Ranger, N. Bundred and M.J. O'Hare *et al*., 2002. Activation of BAD by therapeutic inhibition of epidermal growth factor receptor. J. Biol. Chem., 277: 27643-27650.

Girnita, L., A. Girnita, B. Brodin, Y. Xie, G. Nilsson and J. Landeberg *et al*., 2000. Increased expression of insulin-like growth factor I receptor in malignant cells expressing aberrant p53: functional impact. Cancer Res., 60: 5278-5283.

Guvakova, M. and E. Surmacz, 1999. The activated insulin-like growth factor-I receptor induces depolarization in breast epithelial cells characterized by actin filament disassembly and tyrosine dephosphorylation of FAK, Cas and Paxillin. Exp. Cell Res., 251: 244-255.

Hailey, J., E. Maxwell, K. Koukouras, W.R. Bishop, J.A. Pachter and Y. Wang, 2002. Neutralizing anti-insiulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells. Mol. Cancer Ther., 1: 1349-1353.

Hankinson, S.E., W.C. Willett, G.A. Colditz, D.J. Hunter, D.S. Michaud and B. Derro *et al*. Circulating concentration of insulin-like growth factor-I and risk of breast cancer. Lancet, 351: 1393-1396.

Happerfield, L.C., D.W. Miles, D.M. Barnes, L.L. Thomsen, P. Smith and A. Hanby, 1999. The localization of the insulin-like growth factor receptor I (IGFR-I) in benign and malignant breast tissue. J. Pathol., 155: 1271-1279.

Harman, S.M., E.J. Metter, M.R. Blackman, P.K. Landis and H.B. Carter, 2000. Serum levels of insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3 and prostate-specific antigen as predictors of clinical prostate cancer. J. Clin. Endocrinol. Metab., 85: 4258-4265.

Hellawell, G.O., G.D. Turner, D.R. Davies, R. Poulsom, S.F. Brewster and V.M. Macaulay, 2002. Expression of the type I insulin-like growth factor receptor is upregulated in primary prostate cancer and commonly persists in metastatic disease. Cancer Res., 62: 2942-2950.

Herbert, Y. and R. Thomas, 2000. Role of the insulin-like growth factor family in cancer development and progression. J. Natl. Cancer Inst., 92: 1472-1489.

Hill, M.M. and B.A. Hemmings, 2002. Inhibition of protein kinase B/Akt implication for cancer therapy. Pharmacol. Ther., 93: 243-251.

Hongo, A., C. D'Ambrosio, M. Miura, A. Morrione and R. Baserga, 1996. Mutational analysis of the mitogenic and receptor transforming activities of the insulin-like growth factor-I. Oncogene, 12: 1231-1238.

Huynh, H., R.M. Seyam and G.B. Brock, 1998. Reduction of ventral prostate weight by finasteride is associated with suppression of insulin-like growth factor-I (IGF-I) and IGF-I receptor genes and with an increase in IGF binding protein-3. Cancer Res., 58: 215-218.

Jacobs, S., S. Cook, M.E. Svoboda and J.J. Van Wyk, 1986. Interaction of the monoclonal antibodies alpha IR-1 and alpha-IR-3 with insulin and somatomedin-C receptor. Endocrinology, 118: 223-226.

Jernstrom, H. and E. Barrett-Connor, 1999. Obesity, weight change, fasting insulin, pro-insulin, C-peptide and insulin-like growth factor levels in women with and without breast cancer: The Rancho Bernardo Study. J. Women Health Gend. Based Med., 8: 1265-1272.

Kalebic, T., M. Tsokos and L.J. Helman, 1994. *In vivo* treatment with antibody against IGF-I receptor suppresses growth of human rhabdomyosarcoma and down regulates p34cdc2. Cancer Res., 54: 5531-5534.

Kaleko, M., W.J. Rutter and A.D. Miller, 1990. Overexpression of the human insulin like growth factor-I receptor promotes ligand dependent neoplastic transformation. Mol. Cell Biol., 10: 464-473.

Katia, S., C.M. Maria, N. Giordano, L. Pier-Luigi, L. Stella, B. Stefania, C. Stefania, P. Stefania, Z. Diana, S. Massimo, G.E. Carlos, H. Francesco and P. Piero, 2005. Antitumor activity of the insulin-like growth factor-I receptor kinase inhibitor NVP-AEW541 in musculoskeletal tumors. Cancer Res., 65: 3868-3876.

Kato, H., T.N. Faria, B. Stannard, C.T. Roberts Jr. and D. LeRoith, 1993. Essential role of tyrosine residues 1131,1135 and 1136 of the insulin-like growth factor-I (IGF-I) receptor in IGF-I action. J. Biol. Chem., 268: 2655-2661.

Khandwala, H.M., I.E. McCutcheon, A. Flyvbjerg and K.E. Friend, 2000. The effect of insulin like growth factors on tumorigenesis and neoplastic growth. Endocr. Rev., 21: 215-244.

LeRoith, D., H. Werner, D. Beitner-Johnson and C.T. Roberts Jr, 1995. Molecular and cellular aspect of the insulin like growth factor I receptor. Endocr. Rev., 16: 143-63.

Li, S.L., J. Kato, I.B. Paz, J. Kasuya and Y. Fujita-Yamaguchi, 1993. Two new monoclonal antibodies against the alpha subunit of the human insulin-like growth factor-I receptor. Biochem. Biophys. Res. Commun., 196: 92-98.

Li, S.L., S.J. Liang, N. Guo, A.M. Wu and Y. Fujita-Yamaguchi, 2000. Single-chain antibodies against human insulin-like growth factor-I receptor. Cancer Immunol. Immunother., 49, 243-252.

Long, L., R. Navab and P. Brodt, 1998. Regulation of the Mr 72,000 type IV collagenase by the type-I insulin-like growth factor receptors. Cancer Res., 58: 3243-3247.

Ludwig, D.L., D. Burtrum and D. Lu, *et al*., 2003. A fully human monoclonal antibody to the human IGF-I receptor that blocks ligand-dependent signaling and inhibit growth of multiple human tumors in nude mice. Proc. Am. Assoc. Cancer Res., 44: 761-768.

Lukanova, A., P. Toniolo, A. Akhmed khan, C. Biessy, N.J. Haley and R.E. Shore *et al*., 2001. A prospective study of insulin-like growth factor-I, IGF-binding proteins-1,-2,-3 and lung cancer risk in women. Int. J. Cancer, 92: 888-892.

Ma, J., M.N. Pollak and E. Giovannucci *et al*., 1999. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J. Natl. Cancer Inst., 91: 620-625.

Macauley, V.M., A.J. Salisbury, E.A. Bohula, M.P. Playford, N.I. Smorodinskyand Y. Shiloh, 2001. Down regulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm Kinase. Oncogene, 20: 4029-4047.

Mathieu, M., F. Vignon, F. Capony and H. Rochefort, 1991. Estradiol down-regulates the mannose-6-phosphate/insulin-like growth factor-II receptor gene and induces cathepsin-D in breast cancer cells: A receptor saturation mechanism to increase the secretion of lysosomal proenzymes. Mol. Endocrinol., 5: 815-822.

Mauro, L., D. Sisci, M. Bartucci, M. Salerno, J. Kim and T. Tam *et al*., 1999. SHC-alpha5 beta 1 integrin interaction regulate breast cancer cell adhesion and motility. Exp. Cell Res., 252: 439-448.

Mauro, L., M. Salerno, C. Morelli, T. Boterberg, M.E. Bracke and E. Surmacz, 2003. Role of IGF-I receptor in regulation of cell-cell adhesion: implication in cancer development and progression. J. Cell Physiol., 194: 108-116.

Meyer, G.E., E. Shelde, B. Kim and E.L. Feldman, 2001. Insulin like-growth factor I stimulates motility in human neuroblastoma cells. Oncogene, 20: 7542-7550.

Mira, E., S. Manes, R.A. Lacalle, G. Marques and A.C. Martinez, 1999. Insulin-like growth factor-I triggered cell migration and invasion are mediated by matrix metalloproteinase-9. Endocrinology, 140: 1657-1664.

Muller, M., M. Dietel, A. Turzynski and K. Wiechen, 1998. Antisense phosphorothioate oligodeoxynucleotides, down regulation of the insulin-like growth factor-I receptor in ovarian cancer cells. Int. J. Cancer, 17, 567-571.

Munshi, S., M. Kornienko, D.L. Hall, J.C. Reid, L. Waxman and S.M. Stirdivant *et al.*, 2002. Crystal structure of Apo, unactivated insulin-like growth factor-I receptor kinase: Implication for inhibitor specificity. J. Biol. Chem., 277: 38797-38802.

Nakamura, K., A. Hongo, J. Kodama, Y. Miyagi, M. Yoshinouchi and T. Kudo, 2000. Down regulation of the insulin-like growth I receptor by antisense RNA can reverse the transformed phenotype of human cervical cancer cell lines. Cancer Res., 60: 760-765.

Neuenschwander, S., C.T. Roberts Jr. and D. LeRoith, 1995. Growth inhibition of MCF-7 breast cancer cells by stable expression of an insulin-like growth factor-I receptor antisense ribonucleic acid. Endocrinology, 136: 4298-4303.

Nicholson, K.M. and N. Anderson, 2002. The protein kinase B/Akt signaling pathway in human malignancy. Cell Signal., 14: 381-395.

O'Connor, R., C. Fennelly and D. Krause, 2000. Regulation of survival signals from the insulin-like growth factor-I receptor. Biochem. Soc. Trans., 28: 47-51.

Palmqvist, R., G. Hallmans, S. Rinaldi, C. Biessy, R. Stenling and E. Ribolo *et al.*, 2002. Plasma insulin-like growth factor 1, insulin-like growth factor binding protein-3 and risk of colorectal cancer: a prospective study in northern Sweden. Gut, 50: 642-646.

Pandini, G., F. Frasca, R. Mineo, L. Sciacca, R. Vigneri and A. Belfiore, 2002. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoforms involved. J. Biol. Chem., 277: 39684-39695.

Pautsch, A., A. Zoephel, H. Ahorn, W. Spevak, R. Hauptmann and H. Nar, 2001. Crystal structure of bisphosphorylated IGF-I receptor kinase: insight into domain movements upon kinase activation. Structure (comb), 9: 955-965.

Pavelic, J., L. Pavelic, J. Karadza, S. Krizanac, J. Unesic and S. Spaventi *et al.*, 2002. Insulin-like growth factor family and combined antisense approach in therapy of lung carcinoma. Mol. Med., 9: 955-965.

Pennisi, P.A., V. Barr, N.P. Nunez, B. Stannard and D. LeRoith, 2002. Reduced expression of insulin-like growth factor-I receptors in mcf-7 breast cancer cells leads to a more metastatic phenotype. Cancer Res., 62: 6529-6537.

Peruzzi, F., M. Prisco, M. Dews, P. Salomoni, E. Grassili, G. Romano, *et al.*, 1999. Multiple signaling pathways of the insulin-like growth factor-I receptor in protection from apoptosis. Mol. Cell Biol., 19: 7203-7215.

Pietrzkowski, Z., D. Wernicke, P. Porcu, B.A. Jameson and R. Baserga, 1992. Inhibition of cellular proliferation by peptide analogues of insulin-like growth factor-I. Cancer Res., 52: 6447-6451.

Pietrzkowski, Z., G. Mulholland, L. Gomella, B.A. Jameson, D. Wernicke and R. Baserga, 1993. Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor-I. Cancer Res., 53: 1102-1106.

Probst-Hensch, N.M., J.M. Yuan, F.Z. Stanczyk, Y.T. Gao, R.K. Ross and M.C. Yu, 2001. IGF-1, IGF-2 and IGFBP-3 in prediagnostic serum: Association with colorectal cancer in a cohort of Chinese men in Shanghai. Br. J. Cancer, 85: 1695-1699.

Reinmuth, N, W. Liu, F. Fan, Y.D. Jung, S.A. Ahmad and O. Stoeltzing *et al.*, 2002. Blockade of insulin-like growth factor I receptor function inhibits growth and angiogenesis of colon cancer. Clin. Cancer Res., 8: 3259-3269.

Reiss, K., J.Y. Wang, G. Romano, X. Tu, F. Peruzzi and R. Baserga, 2001. Mechanism of regulation of cell adhesion and motility by insulin receptor substrate-1 in prostate cancer cell. Oncogene, 20: 490-500.

Resnicoff, M., C. Sell, M. Rubin, D. Coppola, D. Ambrose, R. Baserga *et al*., 1994. Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type I insulin-like growth factor receptor. Cancer Res., 54: 4848-4850.

Resnicoff, M., D. Abraham, W. Yutanawiboonchi, H.L. Rotman, J. Kajstura and R. Rubin *et al*., 1995a. The insulin-like growth factor-I receptor protects tumor cells from apoptosis *in vivo*. Cancer Res., 55: 2463-2469.

Resnicoff, M., J.L. Burgaud, H.L. Rotman, D. Abraham and R. Baserga, 1995b. Correlation between apoptosis, tumorigenesis and levels of insulin-like growth factor 1 receptors. Cancer Res., 55: 3739-3741.

Rininsland, F., T.R. Johnson, C.L. Chernicky, E. Schulze, P. Burfeind and J. Ilan, 1997. Suppression of insulin-like growth factor type-I receptor by a triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rat C6 glioblastoma cells. Proc. Natl. Acad. Sci. USA., 94: 5854-5859.

Romano, G., M. Prisco, T. Zanocco-Marani, F. Peruzzi, B. Valentinis and R. Baserga, 1999. Dissociation between resistance to apoptosis and the transformed phenotype in IGF-I receptor signaling. J. Cell Biochem., 72: 294-310.

Rother, K.I. and D. Accili, 2000. Role of insulin receptors and IGF-receptors in growth and development. Pediatr. Nephrol., 14: 558-561.

Ruan, W., L. Powell-Braxton, J.J. Kopchick and D.L. Kleinberg, 1999. Evidences that insulin-like growth factor I and growth hormones are required for prostate gland development. Endocrinology, 140: 1984-1989.

Sachdev, D., S.L. Li, J.S. Hartell, Y. Fujita-Yamaguchi, J.S. Miller and D. Yee, 2003. A chimeric humanized single chain antibody against type-I insulin-like growth factor (IGF) receptor renders breast cancer cells refractory to the mitogenic effect of IGF-1. Cancer Res., 63: 627-635.

Satyamoorthy, K, G. Li, B. Vaidya, D. Patel and M. Herlyn, 2001. Insulin-like growth factor-I induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and beta-catenin pathways. Cancer Res., 61: 7318-7324.

Satyamoothy, K., G. Li, B. Vaidya, J. Kalabis and M. Herlyn, 2002. Insulin like growth factor-I-induced migration of melanoma cells is mediated by inerleukin-8 induction. Cell Growth Differ., 13: 87-93.

Schnarr, B., K. Strunz, J. Ohsam, A. Benner, J. Wacker and D. Mayer, 2002. Down regulation of insulin-like growth factor-I receptor and insulin receptor substrate-I expression in advanced human breast cancer. Int. J. Cancer, 89: 506-513.

Scotlandi, K., S. Benini, P. Nanni, P.L. Lollini, G. Nicoletti, L. Landiuzzi, *et al*., 1998. Blockage of insulin-like growth factor-I receptor inhibits the growth of Ewing's sarcoma in mice. Cancer Res., 58: 4127-4131.

Scotlandi, K., S. Avnet, S. Benini, M.C. Manara, M. Serra and V. Cerisano *et al*., 2002a. Expression of an IGF-I receptor dominant negative mutant induces apoptosis, inhibits tumorigenesis and enhances chemo sensitivity in Ewing's sarcoma cells. Int. J. Cancer, 101: 11-16.

Scotlandi, K., S. Benini, P. Nanni, P.L. Lollini, G. Nicoletti and L. Landiuzzi *et al*., 2002b. Expression of an IGF-I receptor dominant negative mutant induces apoptosis, inhibits tumorigenesis and enhances chemoselectivity in Ewing's sarcoma Cells. Int. J. Cancer, 101: 11-16.

Shaw, L.M., 2001. Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the alpha 6 beta 4 integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion. Mol. Cell Biol., 21: 5082-5093.

Shepherd, P.R., D. Withers and K. Siddle, 1998. Phosphoinositide 3-kinase: The key switch mechanism in insulin signaling. Biochem. J., 333: 471-490.

Sperandio, S., I. DeBelle and D.E. Bredesen, 2002. An alternative nonapoptotic form of programmed cell death. Proc. Natl. Acad. Sci. USA., 97: 14376-14381.

Stattin, P., A. Bylund, S. Rinaldi, C. Biessy, H. Dechaud, U.H. Stenman *et al.*, 2000. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins and prostate cancer risk: A prospective study. J. Natl. Cancer Inst., 92: 1910-1917.

Surmacz, E., C. Sell, J. Swantek, H. Kato, C.T. Roberts Jr., D. LeRoith and R. Baserga, 1995. Dissociation of mitogenesis and transforming activity by C-terminal truncation of the insulin-like growth-I receptor. Exp. Cell Res., 218: 370-380.

Surmacz, E., 2000. Function of the IGF-I receptor in breast cancer. J. Mammary Gland Biol. Neopl., 5: 95-105.

Tanno, S., Y. Mitsuuchi, D.A. Altomare, G.H. Xiao and J.R. Testa, 2001. AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. Cancer Res., 61: 589-593.

Tennant, M.K., J.B. Thrasher, P.A. Twomey, R.H. Drivdahl, R.S. Brinbaum and S.R. Polymate, 1996. Protein and messenger ribonucleic acid (mRNA) for the type I insulin like growth (IGF) receptor is decreased and IGF-II mRNA is increased in human prostate carcinoma compared to benign prostate epithelium. J. Clin. Endocrinol. Metab., 81: 3774-3782.

Torring, N., L. Vinter-Jensen, S.B. Pedersen, F.B. Sorensen, A. Flyvbjerg and E. Nexo, 1997. Systemic administration of insulin-like growth factor-I (IGF-I) causes growth of the rat prostate. J. Urol., 158: 222-227.

Trojan, L.A., P. Kopinski, L.A. Wei Max, A. Glogowska and J. Czarny *et al.*, 2002. IGF-I from diagnostic to triple-helix gene therapy of solid tumors. Acta. Biochem. Pol., 49: 979-990.

Turner, B.C., B.G. Haffty, L. Narayanan, J. Yuan, P.A. Havre and A.A. Gumbs *et al.*, 1997. Glazer insulin like growth factor-I receptor overexpression mediates cellular radioresistence and local breast cancer recurrence after lumpectomy and radiation. Cancer Res., 57: 3079-3083.

Ullrich, A., A. Gray, A.W. Tam, T. Yang-Fong, M. Tsubokawa and C. Collins, 1986. Insulin-like growth factor I receptor primary structure comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J., 5: 2503-2512.

Vadgama, J.V., Y. Wu, G. Datta, H. Khan and R. Chillar, 1999. Plasma insulin-like growth factor –I and serum IGF-binding protein-3 can be associated with the progression of breast cancer and predict the risk of recurrence and the probability of survival in African-American and Hispanic women. Oncology, 57: 330-340.

Vuori, K. and E. Ruoslahti, 1994. Association of insulin receptor substrate-1 with integrins. Science, 266: 1576-1587.

Wen, B., E. Deutsch, E. Marangoni, V. Frascona, L. Maggiorella and B. Abdulkarim *et al.*, 2001. Tryphostin AG1024 modulates radiosensitivity in human breast cancer cells. Br. J. Cancer, 85: 2017-2021.

Werner, H., E. Karnieli, F.J. Rauscher and D. LeRoith, 1996. Wild type and mutant p53 differentially regulate transcription of the insulin like growth factor I receptor gene. Proc. Natl. Acad. Sci. USA., 93: 8313-8323.

White, M.F., 1994. The IRS-signaling system: a network of docking proteins that mediates insulin action. Mol. Cell Biochem., 182: 3-11.

White, M.F., 2002. IRS protein and the common path to diabetes. Am. J. Physiol. Endocrnol. Metab., 283: E413-422.

Wolk, A., C.S. Mantzoros, S.O. Andersson, R. Bergstrom, L.B. Signorello and P. Lagiou *et al.*, 1998. Insulin-like growth factor I and prostate cancer risk: a population based case control study. J. Natl. Cancer Inst., 90: 911-915.

Yu, H., M.R. Spitz, J. Mistry, J. Gu, W.K. Hong and X. Wu, 1999. Plasma levels of insulin-like growth factor-I and lung cancer risk: A case-control analysis. J. Natl. Cancer Inst., 91: 151-156.

Zhang, D. and P. Brodt, 2003. Type-I insulin-like growth factor regulates MTI-MMP synthesis and tumor invasion via PI-3 kinase Akt signaling. Oncogene, 22: 974-982.

Zia, F., S. Jacobs, F. Kull Jr., F. Cuttitta, J.L. Mulshine and T.W. Moody, 1996. Monoclonal antibody alpha IR-3 inhibits non-small cell lung cancer growth *in vitro* and *in vivo*. J. Cell. Biochem. Suppl., 24: 269-275.

Zong, C.S., L. Zeng, Y. Jiang, H.B. Sadowski and L.H. Wang, 1998. Stat 3 plays an important role in oncogenic Ras- and insulin-like growth factor I receptor-induced anchorage-independent growth. J. Biol. Chem., 273: 28065-28072.

Zong, C.S., J. Chan, D.E. Levy, C. Horvath, H.B. Sadowski and L.H. Wang, 2000. Mechanism of STAT 3 activation by insulin-like growth factor-I receptor. J. Biol. Chem., 275: 15099-15105.