Insulin receptor and cancer

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Abstract

The widespread epidemic of obesity and type 2 diabetes has raised concern for the impact of these disorders as risk factors for cancer and has renewed the interest for studies regarding the involvement of hyperinsulinemia and insulin receptor (IR) in cancer progression. Overexpression of IR in cancer cells may explain their increased sensitivity to hyperinsulinemia. Moreover, IR isoform A (IR-A) together with autocrine production of its ligand IGF2 is emerging as an important mechanism of normal and cancer stem cell expansion and is a feature of several malignancies. De novo activation of the IR-A/IGF2 autocrine loop also represents a mechanism of resistance to anticancer therapies. Increasing knowledge of the IR role in cancer has important implications for cancer prevention, which should include control of insulin resistance and hyperinsulinemia in the population and meticulous evaluation of new antidiabetic drugs for their metabolic:mitogenic ratio. We are now aware that several anticancer treatments may induce or worsen insulin resistance that may limit therapy efficacy. Future anticancer therapies need to target the IR-A pathway in order to inhibit the tumor promoting effect of IR without impairing the metabolic effect of insulin.

Endocrine-Related Cancer (2011) 18 R125–R147

Introduction

The insulin receptor (IR) and the IGF1 receptor (IGF1R), both evolved from a common ancestor gene, represent fundamental regulators of glucose metabolism and growth, respectively, in response to nutrient availability. It is now well established that deregulated expression of IGF1R and/or its ligands may have a role in cancerogenesis as well as in cancer progression and resistance to treatments. Recently, the concept that the IR may also be involved in cancer has rapidly evolved as the results of both epidemiological and experimental studies. Epidemiological studies have been stimulated by the growing problem of insulin resistance, mainly due to the worldwide diffusion of lifestyle in western countries. This is associated with epidemic spread of obesity and type 2 diabetes mellitus (T2DM) and consequent chronic subclinical inflammation and metabolic syndrome. A number of epidemiological studies have demonstrated that both obesity and T2DM are important risk factors for a variety of malignancies (Strickler et al. 2001, Kaaks & Lukanova 2002, Vainio et al. 2002, Coughlin et al. 2004, Vigneri et al. 2006) and that hyperinsulinemia is a major cancer risk factor of obese and diabetic patients (Fair et al. 2007, Pisani 2008).

Circulating insulin may affect cancer growth and spread in these patients because insulin resistance is normally restricted to the metabolic pathway, whereas IR signaling pathways leading to cell proliferation and migration are unaffected and even enhanced (Jiang et al. 1999, Cusi et al. 2000). Moreover, malignant cells often overexpress IRs that may reach or exceed expression levels physiologically observed in classical insulin target organs, such as liver or adipose tissue (Papa et al. 1990, Belfiore 2007). Finally, in cancer cells, the functional specificity of insulin/IR signaling is changed because of various mechanisms including altered splicing of the IR gene with the predominant production of IR isoform A (IR-A) that has increased binding affinity for IGFs, enhanced hybrid receptor formation between IR and IGF1R, and autocrine and/or paracrine IGFs production.

This review will focus on molecular mechanisms by which deregulated IRs in malignant cells may contribute to cancerogenesis as well as to cancer progression and resistance to treatment.
Studies linking insulin resistance, circulating insulin, and cancer

Clinical and epidemiological studies

Studies on obese and diabetic patients
Several recent epidemiological studies have found that patients with obesity, T2DM, and metabolic syndrome are at increased risk for a variety of malignancies (Strickler et al. 2001, Coughlin et al. 2004, Vigneri et al. 2006). Compensatory hyperinsulinemia, consequent to insulin resistance, is a major common factor underlying these disorders and is certainly a strong candidate for the increased cancer risk in these patients.

Many recent papers have focused on cancer risk associated with obesity, T2DM, and metabolic syndrome (Calle & Kaaks 2004, Rousseau et al. 2006, Vigneri et al. 2006, 2009) and we refer to them for a detailed description. Limited evidence suggests that patients with T1DM have also an increased cancer risk, possibly because of the effect of exogenous insulin treatment (Zendehdel et al. 2003).

Birth weight and cancer risk
Birth weight is affected by the intrauterine environment and correlates with the level of several growth factors, especially insulin and IGF2, but also IGF1 and sex-steroid hormones. Several lines of evidence support the hypothesis that increased levels of these growth factors in pre- and peri-natal life contribute to the expansion of stem cells, with a consequent increased risk for conversion of these cells into tumor cells later in life (Standaert et al. 1984, McDevitt et al. 2005, Yan et al. 2006, Savarese et al. 2007; see also ‘a possible role for IR-A in cancer stem cell biology and regulation of cell differentiation’ below).

High birth weight has been associated with increased risk for certain cancers, including breast and testicular cancer and childhood leukemia. With regard to breast cancer risk, Xu et al. (2009) recently published a meta-analysis of 18 published studies including 16 424 breast cancer cases. They found that women with a birth weight >4000 g had a higher risk for developing breast cancer than those with birth weight <2500 g (odds ratio (OR)=1.20; 95% confidence interval (CI) 1.08, 1.34). Similar results were found by Park et al. (2008).

A total of 13 epidemiologic studies regarding testicular cancer were recently subjected to meta-analysis. These studies, encompassing 5663 patients, showed a trend for increased testicular cancer in men with a birth weight >4000 g (OR=1.12; 95% CI 1.02–1.22; Michos et al. 2007).

Some evidence suggest that childhood leukemia may be initiated in utero. A meta-analysis of 32 studies examining the association between birth weight and childhood leukemia was recently published (Caughey & Michels 2009) and concluded that high birth weight is associated with an increased risk of overall leukemia and acute lymphoblastic leukemia.

Although these studies do not directly implicate insulin, it is well established that proinsulin/insulin, together with IGFs, is an important determinant of birth weight (Baker et al. 1993, Dornhorst et al. 1994).

Circulating insulin and/or C-peptide and cancer risk
Various studies have directly correlated the level of circulating insulin and/or C-peptide and the occurrence of a particular cancer. A recent meta-analysis of all studies published till 2007 concludes that excess risks of colorectal and pancreatic cancers are associated with increased prediagnostic levels of circulating insulin or C-peptide. No significant association was found among four epidemiological studies on endometrial cancer and C-peptide. Regarding breast cancer, a significant excessive risk was observed only in case–control studies but not in the analyzed four prospective studies (Pisani 2008). Limitations of this analysis include study heterogeneity with the inclusion of retrospective studies and the fact to be based on a single baseline determination of insulin/C-peptide (Fair et al. 2007, Pisani 2008).

In a more recent longitudinal study, carried out in a random sample of 4396 postmenopausal women included in the Women’s Health Initiative Observational Study (WHI-OS), repeated measurements of glucose and insulin at years 1, 3, and 6 were correlated with breast cancer occurrence (Kabat et al. 2009). A group of 1054 women with available blood samples at baseline and at year 3 was also included. It was found that the highest tertile of baseline insulin was associated with a twofold increase in risk in the total population compared with the lowest tertile. No association was seen with glucose levels.

Using the WHI-OS population, Gunter et al. (2009) conducted a case–cohort study of incident breast cancer among non-diabetic women. A total of 835 incident breast cancer case subjects and 816 control subjects were tested for circulating levels of insulin and other variables, including glucose, IGF1, insulin-like growth factor binding protein 3 (IGFBP 3), and estradiol. They found that hyperinsulinemia, and not obesity per se, is an independent risk factor in patients not using hormone therapy (hazard ratio for highest versus lowest quartile of insulin level =2.40; 95% CI=1.30–4.41).
In two recent studies, both performed in women enrolled in the Health, Eating, Activity, and Lifestyle (HEAL) Study and diagnosed with stages I–IIIA breast cancer, the evidence of a link between C-peptide levels or insulin resistance and breast cancer mortality has been reinforced. In the first study, carried out in 604 women (Irwin et al. 2011), a 1 ng/ml increase of fasting C-peptide level was associated with 35% increase in risk of death from breast cancer. The positive association between C-peptide and breast cancer mortality was stronger in certain subgroups, such as women with T2DM, women with a BMI <25 kg/m², women with a higher stage of disease, and women with estrogen receptor-positive tumors. In the second study, performed in 527 women, it was found that increased homeostasis model assessment index (HOMA) and low levels of adiponectin, both markers of insulin resistance, were also positively and independently associated with breast cancer mortality (Duggan et al. 2011).

Overall, these studies support an association between insulin/C-peptide and increased cancer risk and mortality.

Experimental studies

In accordance with epidemiological studies, several experimental models for insulin resistance and hyperinsulinemia have demonstrated a positive correlation between circulating insulin levels and cancer development. Mice and rats affected by streptozotocin- or alloxan-induced diabetes were the first and most widely used animal models linking insulin and cancer. These hyperglycemic and insulin-deficient animals developed lower number of tumors with less aggressive behavior compared with control animals. Insulin administration reverted these effects supporting an intrinsic tumor promoting effect of insulin (Heuson & Legros 1972). These results are in concert with the mitogenic effect of insulin described in azoxymethane-induced colon cancer or precancerous aberrant crypt foci in rats, where injection of medium-acting insulin enhanced tumorigenesis (Tran et al. 1996, Corpet et al. 1997). The stimulatory tumor promoting effect of hyperinsulinemia has also been recently better characterized in engineered animal models of breast cancer. Using a transgenic model (MKR) of type 2 diabetic mice, characterized by severe insulin resistance and hyperinsulinemia in spite of a moderately reduced body adiposity and mild dysglycemia, it was demonstrated that diabetes-related hyperinsulinemia affects mammary gland development and breast cancer progression, independently of obesity and inflammation (Fierz et al. 2010, Novosyadlyy et al. 2010). IR inhibition or insulin level reduction with insulin sensitizers was sufficient to block mammary tumor burden and progression. Similar results were also observed in chemically induced type 1 diabetes mice, where the destruction of pancreatic β-cells suppresses mammary tumor growth, whereas insulin administration reverts this effects (Heuson et al. 1972, Cohen & Hilf 1974, Shafie & Grantham 1981).

In line with these findings, others have shown that increased circulating insulin levels enhance in vivo the growth of LB-T cell lymphoma. In contrast, insulin-deficient diabetic mice and mice fed a low energy diet developed complete resistance to lymphoma growth (Sharon et al. 1993). Yet, intraportal transplantation of pancreatic islets into rats with type 1 diabetes promotes hepatocellular transformation (Dombrowski et al. 1997).

Mechanisms linking hyperinsulinemia and cancer

Increase of IGFs synthesis and/or bioavailability mediated by hyperinsulinemia

Insulin resistance may favor an increased stimulation of the IGF1R by increasing IGF1 bioavailability. At least, 80% of circulating IGF1 is produced in the liver under the control of GH, which also regulates liver production of IGFBP3. Insulin also regulates liver IGF1 production, both directly and also indirectly, by upregulating GH receptors. In addition, insulin also increases IGF1 bioavailability through the downregulation of IGFBP1 and IGFBP2, both inhibit IGF1 actions (Frystyk 2004). In fact, suppressed serum IGFBP1 and increased free IGF1 are observed in the presence of chronic or short-term hyperinsulinemia (Frystyk 2004). IGFBP2 does not respond to acute changes in insulin but increases during chronic low insulin concentrations (Sandhu et al. 2002). Accordingly, low levels of IGFBP2 and high free IGF1 concentrations occur in insulin-resistant patients. The increased IGFs availability and consequent IGF1R overactivation may contribute to cancerogenesis and/or cancer promotion in hyperinsulinemic insulin-resistant patients. The role of IGF1R in cancer has recently been reviewed elsewhere (Pollak et al. 2004, Samani et al. 2007, Werner & Bruchim 2009).

Direct stimulation of IR in tumor cells

Insulin resistance and compensatory hyperinsulinemia are associated with a defect in the proximal part of the insulin signaling network. In particular, studies
and apoptosis and some pathways are more significant. Cell types may use different pathways for proliferation, survival, and metabolism. It is noteworthy that different pathways may be overstimulated in cancer cells because of excessive availability of ligands (insulin and/or IGFs) and/or IR overexpression, and/or altered balance among IR isoforms or because of other, less characterized mechanisms. The predominant activity of these pathways may be, therefore, involved in abnormal stimulation of cell proliferation and/or survival and/or migration.

As mentioned above, the Ras/Raf/MEK/ERK is the key mediator of mitogenic insulin actions transmitting proliferative signals generated by cell surface receptors and cytoplasmic elements to the nucleus. This cascade is activated by insulin following the phosphorylation of IRSs and Shc and the recruitment of Grb/Sos complex. This complex, in turn, triggers the activation of GTPase Ras. Active p21ras recruits a series of intermediate kinases converging on MAPKs, which in turn phosphorylate a number of substrates (SRC1, Pax6, STAT3, cFos, c-myc, and Elk1) involved in the activation of the transcriptional machinery. In this scenario, p21ras plays a key role because it represents a point of convergence for signaling by a number of growth factors. Indeed, the mitogenic effects of growth factors can be amplified by insulin through p21ras. Insulin stimulates the phosphorylation of farnesyltransferase that, in turn, results in increased cellular pool of farnesylated p21ras and its loading with GTP (Goalstone et al. 1997). Farnesylation and subsequent carboxymethylation of p21ras is an essential event for anchoring p21ras to plasma membrane, where it becomes available for subsequent activation by growth factors (Goalstone et al. 1997, Solomon & Goalstone 2001). The PI3K pathway plays the ‘second fiddle’ to MAPK cascade, in terms of cell survival signaling, through various mechanisms including the activation of AKT and of mTOR/p70S6K. After insulin binding to IR and subsequent activation of IRS1, activated PI3K catalyses the production of PtdIns (3,4,5) P3 that, in turn, phosphorylates PDK1 and several downstream enzymes, including AKT and

Therefore, the preferential activation of specific signals depends on many variables that may affect the predominant metabolic or mitogenic effect of IR. Finally, the cross talk and the interactions among different signaling pathways make the IR signaling even more intricate. A schematic representation of IR signaling is shown in Fig. 1. Several recent papers address in a comprehensive way the signaling network of insulin and we refer to them for a more thorough description (Barbieri et al. 2003, Taniguchi et al. 2006, Taguchi & White 2008, Cheng et al. 2010). In this review, we briefly mention the main pathways activated by IR that may be especially relevant to cancer. Some of these pathways may be overstimulated in cancer cells because of excessive availability of ligands (insulin and/or IGFs) and/or IR overexpression, and/or altered balance among IR isoforms or because of other, less characterized mechanisms. The predominant activity of these pathways may be, therefore, involved in abnormal stimulation of cell proliferation and/or survival and/or migration.

Current evidence suggest that direct stimulation of IR has a more important effect in cancerogenesis than previously thought. In cancer patients affected by insulin resistance, the increased levels of circulating insulin combine with the frequent IR overexpression in cancer cells and may result in abnormal stimulation of non-metabolic effects of IR, such as cell survival, proliferation, and migration. These effects are further enhanced by the altered splicing of the IR gene, which generates a predominant expression of IR-A, which has increased affinity for IGFs, especially for IGF2. These mechanisms will be described in more detail in the following paragraphs.

**IR-mediated signaling: relevance to metabolic and non-metabolic effects**

Insulin is a major mediator of important metabolic functions. Indeed, it coordinates and regulates the storage and release of the body’s fuel. Insulin binding to IR triggers the phosphorylation of a complex network of intracellular effectors involved in glucose metabolism and GLUT4 translocation. The PI3K signaling pathway is considered the main cascade responsible for these metabolic actions of insulin. However, insulin may also elicit mitogenic effects that are mainly linked to the activation of the MAPKs cascade, although the PI3K also contributes to IR-mediated cell proliferation and survival. In fact, these two cascades are interconnected and converge on the common mTOR/p70S6K pathway, a major regulator of cell growth, survival, and metabolism. It is noteworthy that different cell types may use different pathways for proliferation and apoptosis and some pathways are more significant than others during the different stages of development. Conducted on obese Zucker (fa/fa) rats, as well as in insulin-resistant patients, have revealed that the PI3K pathway is selectively blunted compared with the MAPK pathway (Jiang et al. 1999, Cusi et al. 2000). Mechanisms accounting for this selective impairment of PI3K/Akt activation mainly involve serine and threonine phosphorylation of the IR β-chain and/or IRS1 phosphorylation at serine residues caused by increased circulating levels of not esterified fatty acids (NEFA) and inflammatory cytokines with consequent activation of intracellular kinases, such as PKCs (Gual et al. 2005). Under these conditions, the attenuation of the PI3K pathway contrasts with the effectiveness of insulin in activating the MAPK pathway, which may even increase (Jiang et al. 1999, Cusi et al. 2000). Therefore, insulin resistance may cause impaired glucose homeostasis in insulin target tissues, while stimulating cell proliferation in other tissues.

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Therefore, the preferential activation of specific signals depends on many variables that may affect the predominant metabolic or mitogenic effect of IR. Finally, the cross talk and the interactions among different signaling pathways make the IR signaling even more intricate. A schematic representation of IR signaling is shown in Fig. 1. Several recent papers address in a comprehensive way the signaling network of insulin and we refer to them for a more thorough description (Barbieri et al. 2003, Taniguchi et al. 2006, Taguchi & White 2008, Cheng et al. 2010). In this review, we briefly mention the main pathways activated by IR that may be especially relevant to cancer. Some of these pathways may be overstimulated in cancer cells because of excessive availability of ligands (insulin and/or IGFs) and/or IR overexpression, and/or altered balance among IR isoforms or because of other, less characterized mechanisms. The predominant activity of these pathways may be, therefore, involved in abnormal stimulation of cell proliferation and/or survival and/or migration.

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PKC. On the basis of the cell context, AKT activates or inhibits several proteins involved in glucose and lipid metabolism (GLUT4, PDE3B, Foxa2, GSK3, and AMPK) but also in cell growth, division, and survival. In fact, AKT phosphorylates the Bcl2 family member BAD involved in the regulation of cell apoptosis. AKT also phosphorylates the transcription factors forkhead proteins (FKHR) and activates the kinase c-Jun N-terminal kinase (JNK), JUN, and p53. AKT also regulates protein translation and cell growth by inhibiting the Tubero sclerosis complex 1 and 2 (TSC1/TSC2) and thus activating mTOR. Phosphorylated IRS1/2 are also able to recruit the Grb2/Sos complex, which triggers the RAS/RAF/MEK/ERK pathway and, as a consequence, the activation of transcription factors (such as Fos and ELK1), mainly involved in cell proliferation.

Another important component responsible for directing metabolic versus mitogenic effects in IR signaling is c-Abl tyrosine kinase. Insulin stimulates c-Abl phosphorylation and FAK dephosphorylation, with consequent metabolic predominance in insulin response on target cells (Frasca et al. 2007). However, in cells with dysfunctional c-Abl, as it may occur in tumor cells, insulin induces FAK phosphorylation and cell proliferation, survival, and migration (Frasca et al. 2007).

The growth promoting effects of insulin could also be mediated by a complex cross talk with β-catenin/Wnt cascade. It is well known that both IR and the canonical Wnt pathways contribute to GSK3 phosphorylation, thus increasing β-catenin stability and its nuclear translocation. Nuclear β-catenin, in turn, binds Lef/Tcf transcription factors and activates target genes involved in cellular proliferation or differentiation programs, such as cyclin D1 (Liang & Slingerland 2003).

S6 Kinase (p70S6K) and eukaryotic initiation factor 4E binding protein (4E-BP1), both positively regulate cell growth (Holland et al. 2004).

Figure 1 Schematic representation of IR signaling. Ligand binding to the α-subunit of IR stimulates the tyrosine kinase activity intrinsic to the β-subunit of the receptor, which in turn phosphorylates several substrates including IRS1/2 and Shc/Grb2/Sos complexes. IRS proteins interact with the regulatory subunit of PI3K leading to the activation of AKT. AKT propagates the IR metabolic effects (glucose uptake, lipogenesis, glycogen synthesis, fatty acid secretion, and gluconeogenesis) by targeting the following substrates: GLUT4, PDE3B, Foxa2, GSK3, and AMPK. However, AKT also represents a crucial node in mediating the growth and survival effects of insulin. Several mediators downstream of AKT are implicated in these effects: the ForkHead transcription factor (FKHR), mouse double minute 2 (Mdm2), Bcl2 antagonists of cell death (BAD), nuclear factor NFκB, Jun N-terminal Kinase (JNK), JUN, and p53. AKT also regulates protein translation and cell growth by inhibiting the Tubero sclerosis complex 1 and 2 (TSC1/TSC2) and thus activating mTOR. Phosphorylated IRS1/2 are also able to recruit the Grb2/Sos complex, which triggers the RAS/RAF/MEK/ERK pathway and, as a consequence, the activation of transcription factors (such as Fos and ELK1), mainly involved in cell proliferation.
A role for IR-A in mediating non-metabolic effects

The Ir gene derives from an ancestor gene, whose duplication has given rise to both the Ir and the Igf1r genes (Brogiolo et al. 2001). The receptors encoded by these two genes have high homology in their structure but different tissue distribution and different biological roles (Nakae et al. 2001). While the IGF1R has the primary role in mediating body growth in response to pituitary GH, the IR has a central role in glucose homeostasis. However, these functional differences are especially evident in postnatal life, whereas IR and IGF1R have partially overlapping effects in prenatal life and in certain pathological conditions (Liu et al. 1993, Accili et al. 1996, Louvi et al. 1997). Of the two isoforms of IR, one, isoform A or IR-A, has a major role in this overlap.

Physiological aspects

Development. Studies carried out on knockout mice indicate that both IR and IGF1R are required for optimal embryonic development and glucose metabolism (Liu et al. 1993, Accili et al. 1996). IR knockout mice are 25% smaller than control mice and show unimpaired glucose metabolism in prenatal life (Accili et al. 1996, Louvi et al. 1997), developing diabetic ketoacidosis only after birth. IR isoform expression is developmentally regulated and IR-A is predominantly expressed in embryo and fetal tissues (Giddings & Carnaghi 1992, Frasca et al. 1999). Therefore, IR-A has been termed ‘the fetal IR isoform’. Recent evidence indicates that IR-A sustains chick embryo development in response to insulin/proinsulin (Morales et al. 1997). In humans, fetal hyperinsulinemia causes macrosomia, whereas insulin deficiency leads to impaired fetal growth, as indicated by infants with congenital diabetes that are small for gestational age. Taken together, these data suggest that IR-A has an important role in mediating growth during prenatal life in response to IGF2 and to proinsulin/insulin (Kaplan 1984).

Postnatal life. In postnatal life, insulin is clearly a key regulator of metabolism by acting at the level of its target tissues, such as liver, adipose tissue, and skeletal muscle, organs that express high IR levels. The IR-B seems to be the most important isoform in mediating these metabolic effects as its relative abundance is ~100% in liver, 80% in adipose tissue, and 50% in muscle (Moller et al. 1989, Seino & Bell 1989, Benecke et al. 1992).

However, IRs have also been found in many other tissues, including brain, heart, kidney, pulmonary alveoli, pancreatic acini, placenta, vascular endothelium, monocytes, granulocytes, erythrocytes, and fibroblasts (Kaplan 1984). These tissues express lower levels than classical target organs and generally have a higher proportion of IR-A, although data are scanty at this regard (Belfiore et al. 2009). The presence of IRs in tissues considered non-canonical targets of insulin metabolic effects suggests that insulin actually elicits non-metabolic effects in these tissues. These effects may include regulation of cell growth, survival, and differentiation, and others. For instance, neuron-specific IR knockout mice have shown a role for IR in the brain in the control of cognitive process, food intake, and reproduction (Kitamura et al. 2003, Okamoto et al. 2004). In pancreatic β-cells, IR plays a role in insulin secretion and possibly in cell survival (Kulkarni et al. 1999). Moreover, specific IR inactivation in endothelial cells has revealed a role for IR in maintaining vascular tone (Vincent et al. 2003). In vitro, the presence of IR-B has a differentiation effect in hematopoietic cells (Sciacca et al. 2003). Both IR-A and IR-B can be involved in these non-metabolic effects on the basis of their expression in different tissues and the specific effects that they mediate.

Functional characteristics of IR-A versus IR-B. As previously mentioned, IR-A seems more involved than IR-B in mediating non-metabolic effects of insulin, at least during development. Some functional differences between the two isoforms with regard to insulin activation have emerged. For instance, the IR-A shows a faster internalization and recycling time (Vogt et al. 1991, Yamaguchi et al. 1993) and may have a less efficient kinase activity (Kosaki et al. 1995). In murine 32D cells, IR-A induces mitogenic and antiapoptotic signals and nuclear translocation of IRS1 (Sciacca et al. 2003, Wu et al. 2003), whereas IR-B induces cell differentiation but not IRS1 nuclear translocation (Wu et al. 2003). The endocytosis of both IR isoforms in living cells has recently been evaluated using confocal microscopy (Giudice et al. 2011). This study has found a higher rate of endocytosis of IR-A compared with IR-B and has correlated these differences with preferential ERK1/2 response to insulin in cells expressing IR-A and preferential Akt response to insulin in cells expressing IR-B.
In pancreatic β cells, the two IR isoforms seem to be located to different membrane subdomains. If confirmed in other cell models, this may explain the divergent intracellular signaling and biological effects of the two isoforms (Leibiger et al. 2001, Uhles et al. 2003).

However, the major functional difference between IR-A and IR-B is represented by the different binding affinity with regard to IGF2 and IGF1. IGF2 binding affinity for IR-A cells is very high (ED$_{50}$ = 2.5 nM), whereas IGF2 binding affinity for IR-B is low (ED$_{50}$ > 100 nM; Frasca et al. 1999). Similarly, the affinity of IGF1 for IR-A is approximately tenfold higher than for IR-B, although 4- to 16-fold lower than that of IGF2 (Yamaguchi et al. 1993, Benyoucef et al. 2007).

The structure, tissue distribution, and ligand affinity of the two IR isoforms, compared with the IGF1R, are shown in Fig. 2. Based on the previously mentioned evidence, we may consider IR-B a specific receptor of insulin predominantly implicated in metabolic effects (Fig. 3A), whereas IR-A is a less specific receptor and also binds IGFs. Because of differences in ligand affinity, kinetics of activation and trafficking, IR-A, likewise IGF1R, mainly favors cell growth, proliferation, and survival (Fig. 3B).

**IR expression and function in cancer cells**

Numerous in vitro and in vivo studies have now clearly established that insulin may affect tumor progression by acting through its own receptor and not by cross talking with the IGF1R, as previously thought (Milazzo et al. 1992, Benoliel et al. 1997, Sciacca et al. 2002). The presence of insulin binding sites was first reported in human breast cancer cells in culture (Osborne et al. 1978). The availability of blocking monoclonal antibodies specific to the IR made it possible to formally demonstrate that insulin mediates growth via the IR in various breast cancer cell lines (Milazzo et al. 1992). IR-A resulted the predominant isoform in most cancer cell lines (Sciacca et al. 1999). IR overexpression either in 3T3 fibroblasts or in MCF10 human breast immortalized cells enhances cell proliferation in response to insulin and induces a ligand-dependent transformed phenotype (Giorgino et al. 1991, Mastick et al. 1994). In endothelial cells infected by Kaposi sarcoma-associated herpes virus, functional IR is essential for transformation (Rose et al. 2007).

A prolonged IR phosphorylation in non-transfected cells can be obtained with certain insulin analogs that have the ability of binding to the IR with a low

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**Figure 2** Structure, tissue distribution, and ligand binding affinity of the IR-A and IR-B and IGF1R. All three receptors (IR-A, IR-B, and IGF1R) show high homology in their molecular structure. L1, large domain 1; CR, cysteine-rich domain; L2, large domain 2; Fn, fibronectin type III domains; TM, transmembrane domain; JM, juxta membrane domain; TK, tyrosine kinase domain; CT, C-terminal domain; ID, insert domain. The hatched fragment on the bottom of the ID-β of IR-B (arrows) is encoded by exon 11 and is present in IR-B but not in IR-A. Below, the predominant tissue distribution of each receptor is shown. At the bottom of the figure, the different ligand binding affinity of each receptor is expressed as IC$_{50}$ values (nM) of $^{125}$I-Insulin displacement (in IR-A and IR-B) or of $^{125}$I-IGF1 displacement by the three ligands, as previously shown (Frasca et al. 1999).
dissociation rate. Therefore, they induce receptor–ligand complexes with increased half-life. The most studied of these analogs, AspB10, was shown to enhance cell proliferation (Ish-Shalom et al. 1997, De Meyts & Shymko 2000) and development of mammary tumors in mice (Drejer 1992). Further evidence that insulin induces growth and other biological effects through the IR have been obtained in various neoplastic cells, which express IRs but non-functional IGF1R levels, such as human myosarcoma cells SKUT1 (Sciaccà et al. 2002), colon cancer cells (LoVo; Jones et al. 2006), and murine T-cell lymphoma cell line (LB cells; Pillemer et al. 1992, Sharon et al. 1993).

However, the presence of a high IR content is not a sufficient prerequisite for responsiveness to insulin. For instance, MDA-MB231 breast cancer cells express high levels of ectonucleotide pyrophosphatase/phosphodiesterase (PC1/ENPP1), which induces selective insulin insensitivity in spite of high numbers of IR-A (Costantino et al. 1993, Belfiore et al. 1996). Although the relative activity of PC1/ENPP1 toward each of the two IR isoforms has not been addressed by specific studies, it has been shown that PC1/ENPP1 is also involved in regulating systemic insulin sensitivity at the level of muscle and liver (Zhou et al. 2009). Further studies need to be performed in order to evaluate whether PC1/ENPP1 expression may be considered a marker of IR activity in cancer cells.

In line with in vitro data, the animal models recapitulating mammary carcinogenesis in diabetic mice strongly support the biological and clinical relevance of IR. Breast tumor tissues extracted from diabetic mice reveal high IR levels and markedly increase IR phosphorylation (Novosyadlyy et al. 2010). IR abundance and its phosphorylation status are positively related to poor survival (Mathieu et al. 1997, Law et al. 2008).

Taken together, these data firmly establish that IRs mediate non-metabolic effects in cancer cells both in vitro and in vivo. The degree of these effects may depend on IR expression levels, IR-A relative abundance, and the integrity of the intracellular machinery that mediate the intracellular IR signaling, as well as the expression levels of endogenous IR inhibitors, such as PC1/ENPP1.

Figure 3 Intracellular signals preferentially activated by insulin binding to the IR-B and by insulin and IGFs binding to either IR-A or IGF1R. (A) Insulin binding to the IR-B induces preferential activation of metabolic signals. This cascade starts with the phosphorylation of IRS1/2 and the activation of PI3K, PI3K, in turn, phosphorylates AKT through PDK1. AKT propagates metabolic signals targeting substrates mostly involved in glucose and lipid homeostasis such as GLUT4, PDE3B, Foxa2, GSK3, and AMPK. Solid lines indicate signaling pathways preferentially activated whereas dashed lines indicate pathways less markedly activated. (B) IR-A and IGF1R activation by insulin and IGFs leads to the predominance of growth and proliferative signals through the phosphorylation of IRS1/2 and Shc proteins. Shc activation leads to the recruitment of Grb2/Sos complex with subsequent activation of Ras/Raf/MEK1 and Erk1/2. This latter kinase translocates to the nucleus and induces the transcription of several genes involved in cell proliferation and survival. Phosphorylation of IRS1/2 induces the activation of the PI3K/PDK1/AKT pathway. Besides its role in metabolic effects, AKT leads to the activation of effectors involved in the control of apoptosis and survival (BAD, Mdm2, FKHR, NFKB, and JNK) and protein synthesis and cell growth (mTOR).
**IR-A activation in cancer by deregulated autocrine and paracrine production of IGFs**

As IR overexpression is a common feature of most malignancies, the question arises as to whether and how it contributes to the activation of the IGF system in addition to IGF1R, which is often concomitantly overexpressed. The answers to these questions are particularly relevant as IGF1R, but not IR, has been chosen as a molecular target of anticancer therapies. In this review, we will describe some molecular mechanisms that may help answer these questions.

**Autocrine/paracrine IGFs production in cancer and receptor binding.** It is well established that both IGF1 and IGF2 are expressed at high levels in a variety of malignancies and function in an autocrine and/or paracrine manner (Samani et al. 2007). In breast and prostate cancer, IGF2 is expressed both by the malignant cells and by the stromal cells adjacent to epithelial malignant cells (Tennant et al. 1996, Rasmussen & Cullen 1998). High levels of IGF2 in the primary tumor have been shown to be predictive of metastases in colorectal (Hakam et al. 1999) and adrenal cortical carcinomas (Gicquel et al. 2001) and to be associated with cancer dedifferentiation in thyroid cancer (Vella et al. 2002). Sarcomas (osteosarcomas, myosarcomas, fibrosarcomas, and Ewing's sarcoma) also express high levels of IGF2 both in vitro and in vivo (Sciacca et al. 2002, Garofalo et al. 2011).

Autocrine IGF1 plays a role in colon (Michell et al. 1997) and prostate cancer (DiGiovanni et al. 2000) and in Ewing's sarcoma (Strammiello et al. 2003). IGF1 may also be produced by cancer stroma (Samani et al. 2007).

As previously mentioned, IGF1 binds with very high affinity not only to IGF1R but also to IR/IGF1R hybrid receptors (EC\textsubscript{50} = 1.0 nM), whereas IGF2 binds to both IGF1R and IR-A with a lower affinity (EC\textsubscript{50} = 10 nM for both receptors). Depending on receptor subtype expression and IGF2 versus IGF1 local availability, a variety of conditions may be envisaged. For instance, if IR-A and IGF1R are concomitantly overexpressed and IGF1 and IGF2 are present in similar molar concentrations, IGF1 will mostly bind and activate its highest affinity receptors (IGF1R and IR/IGF1R), whereas IGF2 will mostly signal through the IR-A, because its binding to the IGF1R is displaced by IGF1. However, if the local IGFs concentration is strongly in favor of IGF2, we can assume that IGF2 can also partially signal through IGF1R and IR/IGF1R hybrids besides binding to IR-A. In the case IGF1 is by far the predominant IGF available to cancer cells, the possibility exists that it may cross talk through the overexpressed low-affinity IR-A, besides binding to its high-affinity receptors (IGF1R and IR/IGF1R hybrids; Fig. 4).

Given that IR-A and IGFs are commonly overexpressed in cancer (Table 1), it seems reasonable to assume that the IR-A, and not the IGF1R, is the principal target of IGF2 in many cancers. In hyperinsulinemic patients, circulating insulin may stimulate both IR isoforms, although, in the presence of a molar excess of IGF2, insulin is expected to mainly stimulate the IR-B.

**Signaling pathways activated by IGF2 binding to IR-A.** Current evidence indicate that signaling pathways and biological effects elicited by IR-A activation in response to IGFs are subtly different than those in response to insulin.

![Figure 4](https://www.endocrinology-journals.org/R133/)

**Figure 4** Schematic representations of hypothetical tumors where both IR isoforms and IGF1R are expressed at equimolar concentrations. (A) In the case that both IGF1 and IGF2 are locally expressed, IGF2 is expected to bind mainly to IR-A because IGF1R and hybrid receptors (HRs) are expected to be saturated by the higher affinity ligand IGF1. (B) In the case that only IGF2 is locally expressed, IGF2 is expected to bind not only to IR-A but also to IGF1R and HRs, all receptors with similar affinity for this ligand. (C) In the case that only IGF1 is locally expressed, IGF1 is expected to bind both to IGF1R and HRs and, possibly also to the lower affinity receptor IR-A. For simplicity, circulating IGFs were not considered. However, they are expected to play a minor role in the presence of high concentrations of locally produced IGFs. The concentration of circulating insulin may widely vary in relation to the degree of insulin sensitivity of the patient.
<table>
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<th>Malignancies reported to overexpress insulin receptor (IR) and IGF1/2 expression</th>
<th>Table 1</th>
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<td><strong>IR</strong></td>
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<td>Breast cancer</td>
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<td>Sarcomas</td>
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<td>Metabolic effect</td>
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In mouse fibroblasts expressing only IR-A (R-/IR-A cells) and not IR-B or IGF1R, IGF2 is a more potent mitogen than insulin itself, although retaining a poor metabolic effect (Frasca et al. 1999). In SKUT1 human rhabdomyosarcoma cells, which lack functional IGF1R and express almost only IR-A, IGF2 stimulates cell chemotaxis more potently than insulin (Sciacca et al. 2002). Moreover, the global profile of gene expression elicited by IGF2 and insulin in R-/IR-A is partially different (Pandini et al. 2003), with IGF2 being more potent than insulin in regulating certain genes (Pandini et al. 2003). Although the signaling pathways responsible of this unique biological response after IR-A activation by IGF2 are not completely understood, some molecular mechanisms are starting to be elucidated.

Using R-/IR-A cells, which represent a suitable model to study the effect of IGFs on IR-A, we found that IGF2 elicits a peculiar signaling pattern characterized by high p70S6K and ERK1/2 response, which reach levels similar to those after insulin; despite, IR-A autophosphorylation is ~50% lower. However, Akt activation is lower after IGF2 than after insulin (Sacco et al. 2009). For explaining these different signaling effects, four possible mechanisms have been considered. First, IGF2 appears to shift the balance of phosphorylation from IRS1 to IRS2 compared to insulin (Sacco et al. 2009). The differential involvement of these two IRS proteins may affect the shift in the balance of p70S6K/Akt activation because of their different trafficking and non-overlapping affinity toward intracellular substrates.

Secondly, more recently, by using a quantitative proteomics approach with R-/IR-A cells, we were able to perform an unbiased analysis of intracellular mediators recruited to tyrosine phosphorylated protein complexes on insulin or IGF2 binding to the IR-A. Data showed that several intracellular mediators were differentially recruited to phosphotyrosine protein complexes associated to the activated IR-A. Interestingly, IGF2 affected the activation of several intracellular mediators more strongly than insulin itself (Morcavallo et al., 2011). Thirdly, IGF2, because of its weaker IR-A stimulation, has a limited ability to activate a PKC-dependent negative feedback involved in signal termination and PKCz, an atypical PKC, may have a role in this mechanism (Sacco et al. 2009). Fourth, differential IR-A and IRS proteins trafficking may strongly contribute to the differential signaling activation between IGF2 and insulin. We recently found that IR-A internalization after IGF2 was much weaker than after insulin and that IRS1 was down-regulated after prolonged insulin exposure but not...
after IGF2 exposure (Morcavallo A, Belfiore A & Malaguarnera R, unpublished observations). Taken together, these data may indicate that the low IR-A phosphorylation occurring after IGF2 binding may differentially affect substrate recruitment to the receptor. It may also protect IR-A and IRS proteins from downregulation, thereby sustaining a more prolonged signaling and more potent mitogenic stimuli.

The signaling and biological effects of IGF1 on binding to the IR-A have been observed only very recently and are, therefore, much less studied. However, it is emerging that IGF1 can stimulate mitogenesis in R-/IR-A cells (Denley et al. 2007) and, similarly to IGF2, affect the balance of intracellular signaling in favor of ERK1/2 and p70S6K activation over Akt activation (Sacco et al. 2009).

A possible role for IR-A in cancer stem cell biology and regulation of cell differentiation

Recently, the findings that most cancers contain malignant stem cells has led to the hypothesis that those malignancies may originate from a small subset of non-differentiated stem/progenitor cells that undergo transformation. Malignant transformation of adult stem cells or progenitors occurs through the dysregulation of the normally tightly regulated self-renewal program. Cancer stem cells have unlimited ability of self-renewal and may drive tumor growth and progression. These cells may also be responsible for resistance to conventional anticancer therapies and for the metastatic spread (Wicha et al. 2006). The molecular mechanisms and the signaling pathways that control stem cell self-renewal remain largely unknown. Recent studies support a role of insulin/IGFs pathway in the biology of progenitor/stem cells. In human embryonic stem (ES) cells, self-renewal and pluripotency depend on IGF2 production by human-ES cell-derived fibroblast-like cells that act as a supportive niche (Bendall et al. 2007). Other research groups have confirmed the important role of IGFs for a variety of stem/progenitor cells (McDevitt et al. 2005, Yan et al. 2006, Stachelscheid et al. 2008). Interestingly, cord levels of IGFs in the newborn are positively associated with the total number of stem cells and with future cancer risk (Savarese et al. 2007). In mouse ES cells, the IR/IGF1R substrate IRS1 is a critical component of the self-renewing program (Rubin et al. 2007).

Recently, we found evidence that IR isoform balance has a critical role in the regulation of stemness and differentiation in stem/progenitor cells from human thyroid. These cells represent a small minority (<1%) of the entire thyroid follicular cell population and can be isolated by culturing them as non-adherent spheres (thyrospheres). In human thyrospheres, we found that IR and IGF1R were markedly overexpressed and with a higher IR:IGF1R ratio compared with primary cultures. The IR:IGF1R ratio was also higher in cancer than in normal thyrospheres. IR-A relative abundance was associated with characteristics of stemness and with cancer: it ranged 65–86% in cancer thyrospheres, 50–65% in normal thyrospheres, and 40–45% in normal thyroid primary cultures or differentiated sphere-derived thyrocytes (Malaguarnera et al. 2011; Fig. 5A).

Both IGF1 and IGF2 were produced at high levels by thyrospheres. However, the IGF1:IGF2 ratio was ~5:1

Figure 5 Expression profile of IR, IGF1R, and IGFs mRNA in thyrospheres and in thyroid differentiating cells. (A) IR (black line) and IGF1R (dashed line) mRNA expression levels in cancer (Ca) and normal thyrospheres (N) and in normal sphere-derived adherent cells grown under differentiating culture conditions (D). The mRNA content of each receptor was measured by qRT-PCR absolute quantification and is expressed as molecules per μg RNA. At the bottom, the relative abundance of IR-A and the IR:IGF1R ratio in either cancer or normal thyrospheres or differentiated sphere-derived cells is indicated, as described previously (Malaguarnera et al. 2011). The proportion of IR-A mRNA was evaluated by PCR analysis and is expressed as percentage. (B) Expression profile of IGF1 (dashed line) and IGF2 (black line) mRNAs in either cancer (Ca) or normal (N) thyrospheres and in normal sphere-derived differentiated cells (D). The mRNA expression levels were measured by qRT-PCR relative quantification. At the bottom, the IGF1:IGF2 ratio is indicated, as described previously (Malaguarnera et al. 2011).
in normal thyrospheres, whereas it was 1:1 in cancer thyrospheres (Fig. 5B).

In cancer thyrospheres IGF1, IGF2 and insulin were able to stimulate sphere volume, but only IGF2 influenced self-renewal, measured as cancer thyr-
osphere ability to give rise to a second generation of spheres (Malaguarnera et al. 2011). Normal thyrospheres could be induced to differentiate. IR and IGF1R, as well as their ligands, sharply declined with differen-
tiation.

Taken together, these data suggest that the IGF2/IR-A pathway plays a critical role in self-renewal of thyroid cancer stem/progenitor cells, although the IGF1/IGF1R pathway is important for processes implicated in sphere volume. Moreover, a high IR-A:IR-B ratio was associated with cell stemness, whereas a decrease in IR-A:IR-B ratio was associated with cell differentiation. Consistent with these data, we previously found that the IR-A:IR-B ratio in thyroid carcinomas increases with tumor dedifferen-
tiation (Vella et al. 2002).

Decrease in the IR-A:IR-B ratio has been associated with changes in cell differentiation in other model systems, as in brown preadipocytes (Entingh et al. 2003), in murine hemopoietic cells (32D; Sciacca et al. 2003), in 3T3-L1 cells (Kosaki & Webster 1993), and in human hepatoblastoma cells (HepG2 cells; Kosaki & Webster 1993, Pandini et al. 2002). Taken together, these data suggest that IR-A overexpression in cancer is associated with de-differentiation and stem-like features. The relationship between IR isoforms and cell differentiation may be bidirectional. In fact, previous data in 32D cells indicate that IR-A transfection preferentially induces mitogenic and antiapoptotic signals, whereas IR-B predominantly induces cell differentiation (Sciacca et al. 2003). The molecular mechanisms by which cell stemness and dedifferentiation are associated with IR-A overexpres-
sion are unclear. Moreover, how the two IR isoforms may have opposite effects on cell differentiation is also unclear and is probably due to subtle different intracellular signaling of the two isoforms (Leibiger et al. 2001).

Therapeutical implications of IR involvement in cancer

Diabetes treatment and cancer risk

As mentioned before, several epidemiological studies and meta-analyses have clearly shown increased cancer risk in T2DM patients and suggest a similar risk also for T1DM patients. This increased risk is attributed to the long-term exposure to elevated insulin concentrations, which in T2DM patients come from insulin resistance, whereas in T1DM, from exogenous insulin administration. Long-standing T2DM may also require insulin treatment. However, exogenous insulins not only do not closely mimic endogenous insulin secretion but also arrive to peripheral tissues and liver at the same time and at similar concentrations (two- to five-fold higher than normal endogenous peripheral levels; Ferrannini & Cobelli 1987). Therefore, diabetes duration, insulin requirement, and co-morbidities may all affect cancer risk.

However, little is known about how antidiabetic drugs may affect cancer risk. These drugs may either increase (e.g. exogenous insulins and sulfonylureas) or decrease (e.g. metformin and thiazolidinediones) circulating insulin levels.

The recent use of insulin analogs has led to some concerns about their potential cancer risk. Currently, three short acting analogs (lispro, aspart, and glulisine) and two long acting analogs (glargine and detemir) are used. The insulin lispro shows an increased affinity for IGF1R and a reduced affinity for IR-A compared with native insulin, but its mitogenic response is similar to that of insulin. The analog aspart is more potent than native insulin in inducing proliferative effects in vitro, whereas the insulin glulisine does not promote any growth advantage to non-malignant mammary cells comparing to the native insulin (Stammberger et al. 2006). Clinical studies on cancer risk in diabetic patients have not focused on the use of these analogs. Regarding to the long-acting insulin analogs (glargine and detemir), some data indicate that glargine or both of them exerts a higher mitogenic effect than native insulin for the preferential activation of ERK pathway, especially in presence of aberrant IR-A expression (Caceres et al. 1994, Kurtzhals et al. 2000, Mayer et al. 2008). Glargine appears to have a higher affinity to IGF1R than native insulin (Kurtzhals et al. 2000, Mayer et al. 2008). However, when administered in vivo, glargine is rapidly metabolized to compounds M1 and M2 with a mitogenic:metabolic ratio similar to that of native insulin (Sommerfeld et al. 2010). The association between insulin glargine and cancer has been addressed in four retrospective clinical trials, three of which indicate that treatment with this type of insulin might be associated with increased cancer risk compared to native insulin. However, these data are controversial and difficult to interpret also for the presence of bias and confounders (Colhoun 2009, Hemkens et al. 2009). If hyperinsulinemia plays a role in increasing cancer risk and progression in diabetic patients, it is reasonable to expect that the secretagogues, sulfonylureas, increasing insulin secretion, and
circulating insulin levels, may also have deleterious effects on cancer. In support of this hypothesis, a retrospective study has shown that T2DM patients exposed to sulfonylureas have a significantly increased risk of cancer-related mortality compared with patients exposed to metformin (Bowker et al. 2006). Yet, it appears that the different classes of sulfonylureas exert differential effects on carcinogenesis (Monami et al. 2007). In particular, glibenclamide was found to be associated with increased incidence of malignancies, whereas gliclazide exerted a protective effect, probably for its antioxidant actions (Monami et al. 2009).

GLP1 analogs and gliptins, both insulin secretion modulators, are the most recent drugs for diabetes treatment, and so far, no data are available on their potential association with cancer risk in diabetic patients.

Biguanides and thiazolidinediones, two classes of antidiabetic drugs, are insulin sensitizers and their use leads to some reduction of insulin levels. Several studies have evaluated the effects of metformin, the only biguanide approved for clinical use. Studies in animal models suggest that the administration of metformin is associated with a reduction in cancer risk (Schneider et al. 2001, Hawley et al. 2003). Evans et al. (2005) have reported a reduction in cancer risk (OD = 0.86) in patients with T2DM taking metformin compared with untreated patients. The protective effect of metformin was also confirmed by different population-based cohort studies demonstrating a significantly reduced risk of cancer-related mortality and cancer incidence in T2DM patients exposed to metformin compared with those treated with sulfonylureas or insulin (Johnson et al. 2002, Bowker et al. 2006, Monami et al. 2009). These data are in accordance with the in vitro results showing that metformin inhibits breast cancer cell proliferation, colony formation, and cell cycle progression (Alimova et al. 2009). The pleiotropic mechanisms of action of metformin explaining its anticancer effects include the ability to reduce insulin resistance, especially at hepatic level, and, as consequence, to decrease hyperinsulinemia and to stimulate the tumor suppressor LKB1 and its downstream effector, AMPK, which reduces the proliferative effects of insulin and IGFs (McCarty 2004, Ruderman & Prentki 2004).

The second class of insulin sensitizers includes the thiazolidinediones. Their potential influence on the association between diabetes and cancer is more controversial. Potential anticancer effects have been reported in several in vitro and in vivo studies (Aiello et al. 2006, Govindarajan et al. 2007, Monami et al. 2008). However, in some cell contexts, thiazolidinediones may induce proliferative responses (Ramos-Nino et al. 2007).

In conclusion, most studies conducted on the possible effects of antidiabetic drugs on cancer risk are difficult to interpret for the presence of bias and confounders and further data need to be collected. So far, clinical and experimental evidence suggests that metformin, either in monotherapy or in combination with other antidiabetic agents, should be recommended to lower cancer risk.

**IR and resistance to anticancer treatments**

Although recent data support an important role of IR in cancer progression and in promoting resistance to both conventional and targeted anticancer treatments, the consideration of IR as a therapeutical target is just emerging. So far, studies aiming to target the IGF axis in cancer have especially focused on the closely related IGF1R, which is considered the receptor of this axis more directly involved in growth and transformation. In fact, IGF1R is also often overexpressed in cancer and is considered to have a dispensable trophic role in adult differentiated cells (Baserga et al. 1997, 2003).

However, the potential importance of IR as a cancer target has being recently re-evaluated after the relatively disappointing results obtained with IGF1R blockade. Many clinical trials with anti-IGF1R blocking antibodies have shown that the activity of these agents is generally modest and only few malignancies show an objective response (Hofmann & Garcia-Echeverria 2005). Responsive neoplasias include ~10% of Ewing’s sarcoma cells and a small subset of non-small cell lung cancers (NSCLC) (Scotlandi 2006, Moro-Sibilot et al. 2010). No biological markers are currently available to identify responsive patients. However, enhanced IR activity seems a common mechanism of resistance.

Increased IR-mediated IRS1/Grb2/Erk/Akt activity (Mur et al. 2002, Fulzele et al. 2007) and enhanced IR-A homodimer formation (Zhang et al. 2007) have been described in cells subjected to IGF1R blockade. Recently, we have demonstrated a role for IR-A in eliciting intrinsic and adaptive resistance to anti-IGF1R therapies in Ewing’s sarcomas. Cells that have developed resistance to specific IGF1R inhibitors, either antibodies or tyrosine kinase inhibitors, showed enhanced IR-A homodimers and/or IGF2 production. These resistant cells thus switch from IGF1/IGF1R to IGF2/IR-A dependency to maintain sustained activation of proliferation, migration, and metastasis (Garofalo et al. 2011).
These findings provide proof of concept that cancers with high basal levels of IR-A and IGF2, especially if with a high IR-A:IGF1R ratio, are expected to be resistant to anti-IGF1R agents. These agents may even exert an adverse effect, as they block the IGF1 feedback at pituitary level and cause GH-induced insulin resistance and hyperinsulinemia, which, in turn, contributes to IR-A activation (Haluska et al. 2006). It is noteworthy that other anticancer therapies, such as antiandrogens and antiestrogens, may also induce insulin resistance and hyperinsulinemia (Bruno et al. 2005, Thurlimann et al. 2005, Redig & Munshi 2010).

For instance, androgen deprivation therapy promotes accumulation of subcutaneous abdominal fat (Smith et al. 2002) and decreased insulin sensitivity (Smith et al. 2001), thus increasing the risk of developing T2DM (hazard ratio = 1.16–1.28; Alibhai et al. 2009, Keating et al. 2010). As insulin resistance and T2DM are associated with an increased risk for cancer and/or a worse cancer prognosis, we may hypothesize that the beneficial effect of various anticancer therapies may be somewhat diminished in patients developing insulin resistance and hyperinsulinemia. These patients might also become at risk for the development of a second malignancy. However, the concern for the adverse metabolic effects of certain anticancer therapies is very recent and further work is needed to define the full oncologic implications of cancer therapy-associated metabolic derangements.

Aberrant IR-A expression may also mediate cancer resistance to other targeted therapies, such as the EGFR inhibitor, gefitinib (Jones et al. 2006), and to mTOR inhibitors (Tamburini et al. 2008).

However, a suitable approach for a target therapy to the IR is still lacking and looks particularly challenging because of the vital role of IR in glucose metabolism. Possible strategies are listed in Table 2. IR-A may be blocked by small molecules with non-selective tyrosine kinase inhibiting activity for both IGF1R and IR, such as BMS-554417 (Haluska et al. 2006). However, this treatment causes marked insulin resistance and hyperinsulinemia, thus the final effect on IR-A overexpressing tumors is uncertain (Haluska et al. 2006). Drugs specifically blocking IR-A generation by interfering with specific RNA splicing factors or by blocking unique components of the IR-A/IGF2 signaling pathway may be effective. However, splicing factors involved in the preferential IR-A generation in cancer cells are still uncharacterized. Intracellular substrates specifically or predominantly activated by this pathway are starting to be identified and characterized (Morcavallo et al., 2011). Insulin analogs with blocking activity for the mitogenic branch of IR-A could, in theory, be designed (Jensen et al. 2007, Jiracek et al. 2010). Another suitable strategy may be represented by the development of antibodies against IGF1 and IGF2. These antibodies have the advantage to inhibit IGF signaling through both the IGF1R and the IR-A pathways. This approach is not new (Miyamoto et al. 2005, Feng et al. 2006) but only very recently has been adequately pursued, with promising inhibitory effects on in vivo growth of IGF1- or IGF2-driven tumors (Gao et al. 2011). The recent finding that both IR and IGF1R belong to the family of dependence receptors also holds promise for the possible development of innovative therapies (Boucher et al. 2010). According to these findings, although ligand-stimulated receptors block apoptosis, unliganded IR and IGF1R exert a permissive effect on cell death. This mechanism does not require tyrosine kinase activity and is probably mediated by unknown partners of unliganded receptors. Hopefully, it could be possible to find a way to turn overexpressed IR-A into proapoptotic molecules. It remains to be ascertained whether anti-IGFs blocking antibodies could unveil this proapoptotic effect of overexpressed receptors by preventing binding. Another possible approach could exploit the peculiarity of IR-A trafficking after IGF2 binding. If significant differences exist between IGF2 and insulin with regard to receptor internalization, recycling, and degradation, it could be hypothesized to use these differences to selectively target IR-A activation and signaling after IGF2. Our recent findings strongly suggest that IR-A trafficking between

**Table 2** Potential insulin receptor isoform A (IR-A) blocking strategies

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<th>Possible approach</th>
<th>Mechanism of action</th>
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<tr>
<td>IGF1R/IR small molecule inhibitors RNA splicing factor inhibitors</td>
<td>Non-selective inhibition of both IGF1R and IR kinase activity Inhibition of IR-A generation</td>
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<tr>
<td>Specific inhibitors of IR-A/IGF2 signaling components IGF1 and IGF2 blocking antibodies</td>
<td>Specific blockade of IR-A/IGF2 downstream signals IGF signaling inhibition</td>
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<tr>
<td>IGF1 and IGF2 blocking antibodies, modulation of unknown mediators of antiapoptotic function of unliganded IR Modulators of IR-A trafficking</td>
<td>Blockade of antiapoptotic function of unliganded IR Specific downregulation/degradation of IR-A</td>
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IGF2 and insulin is indeed different (Morcavallo A, Belfiore A & Malaguarnera R, unpublished observations).

In conclusion, it has become clear that activation of the IGF axis is a crucial aspect of cancer promotion and that aberrant activation of the IR-A/IGF2 loop is the ultimate strategy for cancer cells to benefit of the overactivation of this axis. This loop is particularly difficult to target because of the metabolic function of the IR, which is vital for both normal and cancer cells.

Conclusions and perspectives

The physiological control of glucose metabolism requires phasic insulin secretion in response to nutrients and selective high expression of IR in insulin target organs together with highly specific IR binding to insulin. Whenever one or more than one of these levels of regulation are disrupted, the mitogenic and antiapoptotic effect of IR is enhanced and may promote cancer. Sustained hyperinsulinemia on one side and aberrant IR overexpression and/or altered IR mRNA splicing on the other side are, therefore, associated with cancer initiation and promotion. Overexpression of IR-A and IR/IGF1R hybrid receptors containing IR-A, receptors with broad ligand specificity, makes cancer cells responsive to both insulin and locally produced IGFs.

This emerging scenario has several implications for cancer prevention and treatment. First, systemic disorders, such as obesity, diabetes, and metabolic syndrome, should be considered not only cardiovascular risk factors but also cancer risk factors. Secondly, antidiabetic drugs should ameliorate and not worsen hyperinsulinemia. Thirdly, the mitogenic:metabolic ratio of modified insulins should be extensively tested. Fourth, we should be aware that any therapeutic agent inducing insulin resistance might also increase cancer risk or resistance to anticancer treatments. Future anticancer strategies, aimed to block IGF axis overactivation in cancer, need now to consider new approaches to safely target IR-A activation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This review was supported in part by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC) to A B, and from PRIN-MIUR, grant 2008RKRFBH_005, to A B.
Insulin receptor isoforms and insulin receptor/insulin-like

Bendall SC, Stewart MH, Menendez P, George D,
Vijayaragavan K, Werbowski-Ogilvie T, Ramos-Mejia
V, Rouleau A, Yang J, Bosse M et al. 2007 IGF and FGFind cooperatively establish the regulatory stem cell niche of

Benecke H, Flier JS & Moller DE 1992 Alternatively spliced
B transcription factor.

Benyoucef S, Surinya KH, Hadasschik D & Siddle K 2007
Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and
the alternatively spliced exon 11 sequence to ligand binding and receptor activation. *Biochemical Journal* **403**
603–613. (doi:10.1042/BJ20061709)

Boucher J, Macotela Y, Bezy O, Mori MA, Kriauciunas K &

Bowker SL, Majumdar SR, Veugelers P & Johnson JA 2006
Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* **29** 254–258. (doi:10.2337/dia-care.29.02.06.dc05-1558)

Brogiole W, Stocker H, Ikeya T, Rintelen F, Fernandez R &


Bruno S, Maisonneuve P, Castellana P, Rotmensz N, Rossi S,
Maggioni M, Persico M, Colombo A, Monasterolo F,
663287.E0)

Caceres JF, Stamm S, Helfman DM & Krainer AR 1994
Regulation of alternative splicing in vivo by over-expression of antagonistic splicing factors. *Science* **265**
1706–1709. (doi:10.1126/science.8085156)


Cardillo MR, Monti S, Di Silverio F, Gentile V, Sciarra F &

Caughey RW & Michels KB 2009 Birth weight and
cancer. *American Journal of Epidemiology* **169**
discussion 57–60.

Denley A, Carroll JM, Brierley GV, Cosgrove L, Wallace J,
Forbes B & Roberts CT Jr 2007 Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors. *Molecular and Cellular


Giudice L, Leskow FC, Arndt-Jovin DJ, Jovin TM & Jares-Erijman EA 2011 Differential endocytosis and...


Kosaki A, Pillay TS, Xu L & Webster NJ 1995 The B isoform of the insulin receptor signals more efficiently than the A isoform in HepG2 cells. Journal of Biological Chemistry 270 20816–20823. (doi:10.1074/jbc.270.35.20816)

Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA & Kahn CR 1999 Tissue-specific knockout of the insulin receptor (Igf1r).


Liou A, Baker J, Perkins AS, Robertson EJ & Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75 59–72. (doi:10.1016/S0092-8674(05)80084-4)


Nickerson T, Chang F, Lorimer D, Smeekeens SP, Sawyers CL & Pollak M 2001 In vivo progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR). *Cancer Research* **61** 6276–6280.


receptors have different biological characteristics depending on the insulin receptor isoform involved. *Journal of Biological Chemistry* **277** 39684–39695. (doi:10.1074/jbc.M202766200)


Sciacca L, Mineo R, Pandini G, Murabito A, Vigneri R & Belfiore A 2002 In IGF-I receptor-deficient...
leiomyosarcoma cells autocrine IGF-II induces cell invasion and protection from apoptosis via the insulin receptor isoform A. *Oncogene* **21** 8240–8250. (doi:10.1080/0165121010206058)


Tennant MK, Thrasher JB, Twomey PA, Drivdahl RH, Birnbaum RS & Plymate SR 1996 Protein and messenger ribonucleic acid (mRNA) for the type 1 insulin-like growth factor (IGF) receptor is decreased and IGF-II mRNA is increased in human prostate carcinoma compared to benign prostate epithelium. *Journal of Clinical Endocrinology and Metabolism* **81** 3774–3782. (doi:10.1210/jc.81.10.3774)


Tran TT, Medline A & Bruce WR 1996 Insulin promotion of colon tumors in rats. Cancer Epidemiology, Biomarkers & Prevention 5 1013–1015.


Werner H & Bruchim I 2009 The insulin-like growth factor-I receptor as an oncogene. Archives of Physiology and Biochemistry 115 58–71. (doi:10.1080/13813450902783106)


Received in final form 8 May 2011
Accepted 16 May 2011
Made available online as an Accepted Preprint 16 May 2011