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REVIEW

Tumor suppressors govern insulin-like growth factor signaling pathways: implications in metabolism and cancer

H Werner

Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

The insulin-like growth factor (IGF) axis mediates growth, differentiation and developmental processes, and is also involved in control of metabolic activities. Deregulation of IGF axis expression and action is linked to a number of pathologies, ranging from metabolic disorders to growth deficits and cancer development. Activation of the IGF signaling pathway is a crucial prerequisite for malignant transformation. In addition, overexpression of the IGF-1 receptor (IGF-1R) constitutes a typical hallmark of most types of cancer. A series of tumor suppressors have been identified whose mechanisms of action involve transcriptional suppression of the *IGF-1R* gene. These tumor suppressors include the p53/p63/p73 family, breast cancer gene-1, von-Hippel Lindau protein, Wilms' tumor-1 and others. Comprehensive analyses have identified a complex bidirectional interplay between the IGF and tumor-suppressor signaling pathways. These interactions are of major importance in terms of cancer development and may also predict responsiveness to IGF-1R-targeted therapies. Furthermore, the insulin/IGF system has a pivotal role in the regulation of cancer cell metabolism. Deregulation of IGF axis components by mutated tumor-suppressor proteins may lead to metabolic perturbations, with ensuing pathological consequences.
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The insulin/IGF axis: a network of ligands, receptors and binding proteins

The insulin-like growth factors (IGF-1, IGF-2) constitute a network of cellular and secreted proteins with vital functions in multiple biological processes (LeRoith and Yakar, 2007; Maki, 2010). Since their discovery in the mid-1950s by Salmon and Daughaday (1957), the IGFs have attracted huge scientific attention. Interest in this family of hormones, cell-surface receptors, and circulating and membrane-bound IGF-binding proteins (IGFBPs) nurtures mainly from the recognition that the IGF signaling pathways are involved in a myriad of pathophysiological processes with ample clinical relevance in the areas of endocrinology, pediatrics, ageing research, oncology and others (LeRoith *et al*., 2001; Baserga *et al*., 2003; Samani *et al*., 2007; Pollak, 2008; Werner and Bruchim, 2009).

IGF-1, which was initially identified by its ability to mediate the effects of growth hormone (GH) on cartilage sulfation and, in a broad sense, on longitudinal growth, is produced mainly by the liver, although many organs possess the biosynthetic machinery necessary to produce the hormone at various levels. It has been classically accepted that the locally produced IGF-1 is mainly involved in autocrine–paracrine types of activities, whereas circulating (that is, liver-produced) IGF-1 mediates endocrine activities (LeRoith *et al*., 2001). Similarly, the concept that insulin receptor (IR) activation (mainly by insulin) leads primarily to metabolic activities while IGF-1 receptor (IGF-1R) activation (mainly by IGF-1 or IGF-2) leads to proliferative and differentiative events, was the prevalent belief for almost 40 years (Nakae *et al*., 2001). These dogmas have been challenged in recent years due, in part, to a number of technological and scientific breakthroughs (Yakar *et al*., 2005; Werner and Bruchim, 2010; Belfiore and Malaguarnera, 2011). These advances included: (1) the availability of animal models with organ-specific disruptions (or overexpression) of particular ligands or receptors; and (2) the introduction of genomic, proteomic and metabolomic methodologies that allow global analyses of massive amounts of data. These (and other) developments permit a comprehensive dissection of the growth and metabolic activities of the insulin/IGF axis, and are having a profound impact on our ability to understand the biology of the IGF system in an integrated manner.

The present review is aimed at evaluating the interplay between the IGF-1 signaling pathways and classical cancer genes. Evidence will be presented showing an intimate bidirectional cross-talk between the IGF-1R and oncogene/tumor-suppressor pathways, with a substantial impact on both metabolic and proliferative events. In addition, data illustrating a novel role of the *IGF-1R* gene as a downstream target for tumor-suppressor action will be described. A critical analysis of this paradigm may shed light on basic and clinical questions.

Correspondence: Professor H Werner, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel.
E-mail: hwerner@post.tau.ac.il
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An epidemiological link between the insulin/IGF axis and cancer

Reports published in 1998 identified, for the first time, a link between serum IGF-1 levels and breast and prostate cancer risk. These studies, based on the Nurse's and Physician's Health Studies, demonstrated that the relative risk of breast cancer in premenopausal women in the upper tertile of IGF-1 values was 4.6 (Hankinson *et al*., 1998). Similarly, the relative risk of prostate cancer in men in the upper quartile of IGF-1 values was 2.4 (Chan *et al*., 1998). Of importance, IGF-1 levels were measured an average of 7 years before diagnosis of the disease. These reports had a huge impact in the field and were followed soon by studies from numerous groups worldwide. A meta-regression analysis by Renehan *et al*. (2004) identified 21 eligible studies, including 3609 cases and 7137 controls. Their study concluded that high serum levels of IGF-1 are correlated with increased risk of common cancers, but associations seem to be smaller than those reported in earlier studies. Furthermore, the correlations seem to vary between cancer sites.

A converse perspective to the epidemiological link between endocrine IGF-1 and cancer risk was recently provided by the analysis of individuals with Laron syndrome (LS), a form of dwarfism caused by mutation of the GH receptor gene (Laron, 2004). LS patients exhibit GH insensitivity and, consequently, *congenital IGF-1 deficiency*, with undetectable levels of circulating IGF-1. In a recent worldwide survey of patients with LS and other entities within the spectrum of congenital IGF-1 deficiencies (for example, isolated GH deficiency, GH-releasing hormone receptor mutations, congenital multiple pituitary hormone deficiency) none of the 230 LS patients included developed cancer (up to the age of $\sim$85), whereas family members (heterozygotes for the mutation) were affected similarly to the general population ($\sim$10–20% cancer incidence) (Steuerman *et al*., 2011). Similar trends were seen with the other pathologies. Although based on a small population (though amounting, by different estimates, to $\sim$30–50% of the total number of patients worldwide), this study is consistent with the notion that homozygous congenital IGF-1 deficiency confers protection against future development of cancer. Along this same line, a study based on an Ecuadorian cohort of LS patients showed that GH receptor deficiency was associated with a reduction in pro-aging signaling and cancer (Guevara-Aguirre *et al*., 2011). In addition, the GH receptor defect was correlated with low insulin levels and high insulin sensitivity, leading to a reduction in diabetes risk. Other investigators, however, reported that untreated LS patients developed insulin resistance, which leads later in life to the development of glucose intolerance and type 2 diabetes (Laron and Weinberger, 2004).

The epidemiological link between circulating values of insulin/IGF-1 and cancer must be dealt under the broader umbrella of the correlations between nutrition, obesity and cancer, also known as the *insulin–cancer hypothesis* (Renehan *et al*., 2006; Dossus and Kaaks, 2008). This hypothesis postulates that chronic hyperinsulinemia, a typical hallmark of diabetes, is one of the leading factors responsible for the obesity–cancer connection. Numerous cellular and circulating factors are involved in the biochemical chain of events leading from hyperinsulinemia and insulin resistance to increased cancer risk and, eventually, tumor development. The IGFs are key players in the complex biochemical network linking nutrition, obesity and cancer.

IGF-1R activation is a pre-requisite for malignant transformation

The IGF-1R exhibits a very potent anti-apoptotic activity in comparison with most other growth factor receptors described (Harrington *et al*., 1994; Resnicoff *et al*., 1995; O'Connor *et al*., 1997). This activity confers upon IGF-1R-expressing cells enhanced survivability, a key hallmark of cancer cells. Seminal studies from the laboratory of Renato Baserga provided evidence that cells derived from *IGF-1R* 'knock-out' embryos (the total deficiency of IGF-1R is a lethal condition), with a few exceptions, do not undergo malignant transformation when exposed to oncogenes (Sell *et al*., 1993, 1994. Hence, these early studies were consistent with the notion that IGF-1R expression and/or activation are fundamental pre-requisites for cancer development. It is important to realize that IGF-1R, *per se*, is neither genotoxic nor transforming. In other words, activation of the IGF-1R by IGF-1 is not an oncogenic event. IGF-1, however, is an important *progression factor* necessary for cell cycle progression *after* cell exposure to a *competence factor* (for example, platelet-derived growth factor, fibroblast growth factor). Once an oncogenic event has occurred (that is, a first *hit*), cell survival of *already* transformed cells is heavily dependent on IGF-1 action. Unlike IGF-1, overexpression of IGF-2 has been linked to the etiology of a number of overgrowth syndromes (for example, Beckwith–Wiedemann Syndrome) and cancers (for example, Wilms' tumor, rhabdomyosarcoma) (Bentov and Werner, 2004). In this context, it was shown that the initial proliferative switch in oncogene-induced transformation was correlated with focal activation of IGF-2 (Christofori *et al*., 1994).

In agreement with its central role in neoplasia, the IGF-1R emerged in recent years as a promising therapeutic target. Targeting modalities embrace the use of IGF-1R monoclonal antibodies (as monotherapy or in combination with other antibodies and/or classical therapies) as well as small molecular weight tyrosine kinase inhibitors (Scotlandi and Picci, 2008; Yuen and Macaulay, 2008; Bruchim *et al*., 2009). Given the structural similarity between IGF-1R and IR and in view of their overlapping pathways, the probability of 'knocking-down' the IR (with ensuing metabolic impairment) when applying anti-IGF-1R therapies became a matter of concern (Gualberto and Pollak, 2009). On the other hand, the recognition that the IR (and, in particular, the IR-A isoform) is an important player in breast cancer etiology might imply that dual (for example, IR and IGF-1R) targeted therapy offers

obvious advantages (Belfiore and Frasca, 2008). This issue has not yet been resolved.

Overexpression of IGF-1R is a common theme in human cancer: the rule and the exceptions

Clinical and experimental studies conducted since the 1980s have provided undisputable evidence that most tumors display enhanced IGF-1R concentrations and express high IGF-1R mRNA levels (Mitsiades *et al*., 2004; Werner, 2009). These augmented levels reflect a reversal to more primitive, less differentiated, developmental stages (usually associated with very high IGF-1R and IGF-2 mRNA levels) (Bondy *et al*., 1990). The dogma that evolved postulated that, as mentioned above, IGF-1R overexpression is a *sine qua non* prerequisite for oncogenic transformation. The charm of this hypothesis resided in the fact that enhanced IGF-1R levels and IGF-1/IGF-2 signaling were considered vital factors, indispensable for the cell in order to adopt proliferative/oncogenic pathways. However, IGF-1R overexpression, *per se*, does not necessarily reflect the existence of a cancerous phenotype. Thus, low IGF-1 concentrations (for example, in LS serum) may upregulate IGF-1R levels in erythrocytes without evidence of cancer (that is, without IGF-1R activation) (Eshet *et al*., 1993). On the other hand, elevated circulating insulin and IGF-1 can downregulate *IGF-1R* gene expression. Furthermore, steroid hormones (for example, estrogens, androgens) as well as other growth factors (for example, FGF, PDGF) were shown to enhance IGF-1R production under physiological conditions (Hernandez-Sanchez *et al*., 1997; Pandini *et al*., 2005; Maor *et al*., 2006).

Hence, the prevailing notion that *all* transformed cells overexpress the IGF-1R and, by extension, that IGF-1R overexpression *equals* malignancy is, obviously, an overgeneralization (Werner and Roberts, 2003). Whereas IGF-1R overexpression is a common feature of solid pediatric, hematological, brain, renal and other tumors, the situation in adult epithelial tumors is more complex and additional factors must be taken into consideration, including the stage of the disease, steroid hormone status, and the activation state of multiple signaling molecules. In breast tumors, for example, IGF-1R is expressed at high levels in control mammary tissue and in well- and moderately differentiated breast carcinoma, but at significantly lower levels in poorly differentiated cancers (Schnarr *et al*., 2000). In prostate cancer, similarly, a marked reduction in IGF-1R levels was seen during transformation of prostate epithelial cells from a benign to a metastatic state (Plymate *et al*., 1997). Total loss of expression was reported in bone metastases (Chott *et al*., 1999). However, other studies do not support the concept of reduced IGF-1R levels in metastatic cancer (Hellawell *et al*., 2002). The reduction in IGF-1R levels in tumors that are heavily dependent on steroid hormones (for example, breast, prostate) may reflect the fact that both androgens and estrogens stimulate *IGF-1R* gene expression (Maor *et al*., 2006; Schayek *et al*., 2010b). Hence, steroid hormone independence at advanced cancer stages may lead to a reduction in growth factor receptor expression. Finally, activation of the IGF-1R signaling pathway (that is, phosphorylation of the receptor tyrosine kinase domain and downstream molecules) is regarded as a fundamental requirement in transformation. Therefore, the relevance and implication of IGF-1R expression in cancer must be evaluated in a broader context, including analyses of signaling pathways (for example, IRS-1, *ras-raf*-mitogen-activated protein kinase, Akt/protein kinase-B) and interactions with cancer genes. The reverse paradigm (that is, that augmented IGF-1R expression in cancer is a *consequence* of the neoplastic phenotype) is, similarly, a biologically plausible theory that merits thorough consideration.

In the context of IGF-1R expression, it is pertinent to question what are the genetic and molecular mechanisms responsible for pathological IGF-1R deregulation. Of interest, *IGF-1R* mutation is a very rare event that has been reported only in a number of cases in heterozygote form (Klammt *et al*., 2008, 2011; Kruis *et al*., 2010; Wallborn *et al*., 2010). Moreover, these mutations were not associated with neoplasia but rather with growth retardation. In a cohort including 42 patients with unexplained intrauterine growth retardation and subsequent growth failure, a girl was identified who was a compound heterozygote for point mutation in exon 2 of the *IGF-1R* gene that altered the amino acid sequence to Arg108Gln in one allele and Lys115Asn in the other. Fibroblasts cultured from the patient had decreased IGF-1R binding and phosphorylation (Abuzzahab *et al*., 2003). In another cohort including 50 children with short stature and high IGF-1 levels, a boy was identified with a nonsense mutation, Arg59STOP that reduced IGF-1R number.

In the next sections, we shall discuss some of the mechanisms associated with enhanced IGF-1R expression in cancer. A schematic diagram of the *IGF-1R* regulatory region is presented in Figure 1. Elucidation of the *IGF-1R* promoter structural features and identification of *IGF-1R*-promoter-binding transcription factors and mechanisms proved important in order to understand its modulation by oncogenes and tumor suppressors (Sarfstein *et al*., 2010).

Wild type, but not mutant, p53 suppresses *IGF-1R* gene transcription

An illustrative paradigm of the interaction between the IGF-1 signaling pathway and cancer genes is provided by the interplay between p53 and the *IGF-1R* gene (Levine *et al*., 2006). p53 is a tumor-suppressor gene product that usually accumulates in the cell in response to DNA damage, and which constitutes the most frequently mutated molecule in human cancer (Levine, 1997). When hyperphosphorylated, wild-type p53 arrests cell cycle progression at the G_1 phase, hence enabling damaged DNA to be repaired before the replicative phase. p53 can, alternatively, elicit an apoptotic program and multiple target genes of p53

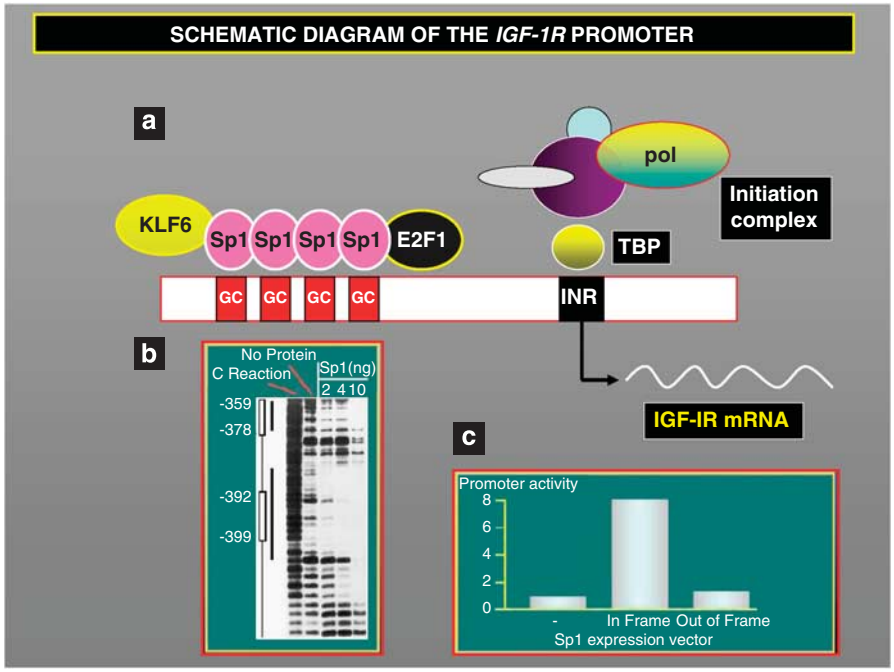

Figure 1 Schematic diagram of the *IGF-1R* promoter. (**a**) The *IGF-1R* regulatory region is highly GC-rich and lacks canonical TATA or CAAT motifs, two regulatory elements that are required for efficient transcription initiation of most eukaryotic genes. Accurate transcription of the *IGF-1R* gene is directed from an 'initiator' (INR) sequence, a control element that is present in gene promoters that are highly regulated during differentiation and development. The 'initiator' element is able to assemble a functional transcription complex in the absence of a TATA box. Recent proteomic analyses linked to mass spectroscopy identified a series of nuclear proteins that differentially bind to the *IGF-1R* promoter in estrogen receptor (ER)-positive and ER-depleted breast cancer cells (Sarfstein *et al*., 2010). A key transactivator of the *IGF-1R* gene is Sp1. Sp1 is a ubiquitous zinc-finger nuclear protein that stimulates transcription from a group of RNA polymerase II-dependent promoters. (**b**) DNaseI foot printing assays have identified specific Sp1 binding to consensus GC-boxes in the proximal promoter region. (**c**) Coexpression of *in-frame*, but not *out-of-frame*, Sp1 in *Drosophila* Schneider cells (devoid of endogenous Sp1) led to a marked activation of the promoter. As described in the text, the mechanisms of action of a number of tumor suppressors, including BRCA1 and VHL, involve physical and functional interactions with Sp1. Additional *trans*activators of the *IGF-1R* gene include the Krüppel-like factor-6 (KLF6) and E2F1 (Rubinstein *et al*., 2004; Schayek *et al*., 2010a).

have been identified (Aylon and Oren, 2011; Tang *et al*., 2011). The functional interactions between p53 and the *IGF-1R* gene were examined by means of coexpression experiments in osteosarcoma and rhabdomyosarcoma cell lines using wild-type or mutant p53 expression vectors along with an *IGF-1R* promoter-luciferase reporter (Figure 2). Wild-type p53 suppressed the activity of the *IGF-1R* promoter by ~75–90% whereas co-transfection of tumor-derived, mutant versions of p53 strongly enhanced promoter activity (Werner *et al*., 1996; Idelman *et al*., 2003). The effect of p53 was mediated at the level of transcription, as shown by *in vitro* transcription assays. In addition, wild-type p53 decreased the IGF-1-induced tyrosine phosphorylation of IGF-1R and IRS-1, while mutant p53 stimulated phosphorylation (Ohlsson *et al*., 1998). These results support the view that, at least part of, the effects of wild-type p53 on apoptosis and cell cycle arrest are mediated *via* suppression of the *IGF-1R* promoter. Lack of inhibition, or even stimulation, of the *IGF-1R* gene by mutant p53 may accelerate tumor growth and inhibit apoptosis, hence providing an increased survival capacity to malignant cells.

Although the mechanism for transcriptional suppression of *IGF-1R* by p53 is not fully understood, results of electrophoretic mobility shift assays suggest that wild-type p53 can bind the TATA box-binding protein, thus preventing this protein from binding to the initiator element and assembling a functional initiation complex at the *IGF-1R* promoter (Figure 2). An additional mechanism of action of p53 involves its interaction with zinc-finger protein Sp1, a potent transactivator of the *IGF-1R* promoter. Importantly, p53 has been shown to modulate additional components of the IGF axis. Thus, the expression of IGF-2 transcripts was reduced by wild-type p53 (Zhang *et al*., 1996) whereas the activity of the *IGFBP3* gene was stimulated by wild type, but not mutant, p53 (Buckbinder *et al*., 1995). Given that IGFBP3 is an inhibitor of mitogenic signaling by IGFs, it may be inferred that p53 regulates the IGF system at multiple levels, including availability of IGF ligands and activity of the *IGF-1R* promoter.

Evidence in support of a bidirectional interplay between the IGF-1 and p53 signaling pathways was provided by studies showing that IGF-1 induces p53 degradation in an Mdm2-dependent manner *via* the p38 MAPK pathway in response to DNA damage (Heron-Milhavet and LeRoith, 2002). Mdm2 is an ubiquitin ligase of primary importance in regulation of p53 activity (Lakin and Jackson, 1999). Mdm2 was shown

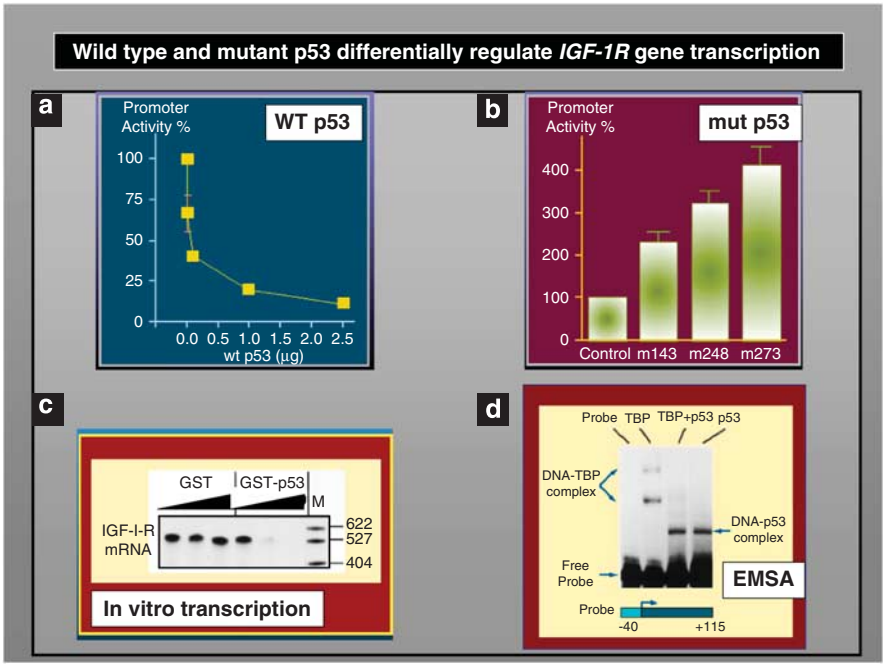

Figure 2 Wild-type and mutant p53 differentially regulate *IGF-1R* gene transcription. (**a**) Coexpression of wild-type p53 along with an *IGF-1R* promoter-luciferase reporter construct in osteosarcoma-derived Saos-2 cells (devoid of endogenous p53) led to a dose-dependent repression of *IGF-1R* promoter activity (Werner *et al*., 1996). (**b**) Co-transfection of tumor-derived mutant versions of p53 (mutated at codons 143, 248 and 273) resulted in marked transactivation of the *IGF-1R* promoter. (**c**) *In vitro* transcription assays were performed by incubating a HeLa whole cell extract and increasing amounts (25, 75, 150 ng) of purified glutathione *S*-transferase (GST) or GST-p53 protein, along with a purified DNA template extending from nt −476 to +640 of the *IGF-1R* promoter. Results of *in vitro* transcription assays showed that p53 suppressed *IGF-1R* gene expression at the level of transcription. (**d**) Electrophoretic mobility shift assays (EMSA) were performed using a ^{32}P-labeled DNA fragment extending from −40 to +115 (that is, encompassing the 'initiator' element). Addition of purified TATA-binding protein (TBP, lane 2) generated two retarded bands. The formation of the *IGF-1R* promoter-TBP complexes was abolished by addition of purified GST-p53 (lane 3). In addition, incubation of GST-p53 with the DNA fragment generated a nonspecific band, both in the presence or absence of TBP.

to physically associate with the IGF-1R and to induce receptor ubiquitination and degradation (Girnita *et al*., 2003). Convergence of the IGF-1 and p53 signaling pathways was also suggested by studies indicating that Mdm2 constitutes a substrate for protein kinase Akt (Zhou *et al*., 2001). Akt itself is activated by IGF-1 *via* activation of the IGF-1R. Furthermore, a number of biological actions of IGF-1 depend on the presence of an intact p53. For example, IGF-1 was shown to stimulate Kruppel-like factor-6, a zinc-finger tumor suppressor inactivated in prostate and other types of cancer, expression in cells with normal, but not inactivated, p53 (Bentov *et al*., 2008).

Finally, the identification of the *IGF-1R* gene as a downstream target for p53 action provides a biologically plausible paradigm with potentially important implications in cancer biology (Figure 3). Is this prototype shared by other members of the p53 family? The p63/p73 gene family displays a large structural heterogeneity that rules out any generalization regarding their roles in cancer (Yang and McKeon, 2000; Irwin and Kaelin, 2001). However, similarly to p53, a number of p63/p73 isoforms were shown to induce a dose-dependent decrease in endogenous IGF-1R levels, suggesting that the *IGF-1R* gene constitutes also a physiologically relevant target for p63/p73 action (Nahor *et al*., 2005). Moreover, all isoforms assayed suppressed *IGF-1R* promoter activity. The ability of additional tumor suppressors to control *IGF-1R* gene expression will be examined next.

Mutant breast cancer gene-1 (BRCA1)-associated breast tumors express elevated IGF-1R levels: molecular basis and clinical implications

The breast and ovarian cancer susceptibility genes (*BRCA1, BRCA2*) have been identified as tumor suppressors whose mutation correlated with the appearance of breast and/or ovarian cancer at young ages (Miki *et al*., 1994; Narod, 2010). BRCA1 is involved in several biological pathways, including DNA damage repair, apoptosis, cell growth and gene transcription (Boulton, 2006). To evaluate the interactions between BRCA1 and the IGF system, co-transfections were performed in breast cancer-derived cell lines using a BRCA1 expression vector along with an *IGF-1R* promoter-luciferase reporter. Consistent with its tumor-suppressor role, BRCA1 expression resulted in significant reductions in endogenous IGF-1R levels and *IGF-1R* promoter activity (Maor *et al*., 2000) (Figure 4). On the other hand, a mutant *BRCA1* gene encoding

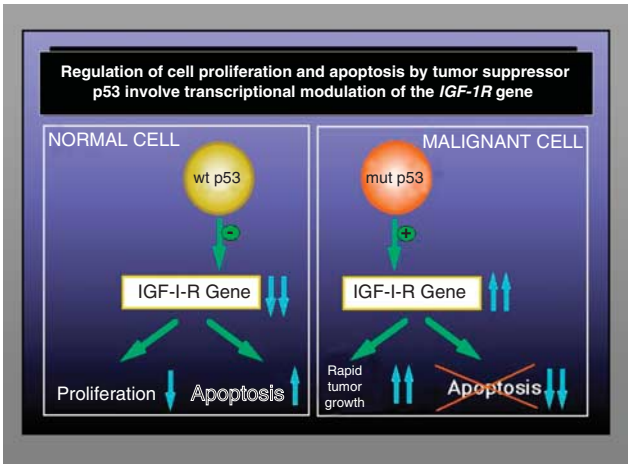

Figure 3 Regulation of cell proliferation and apoptosis by tumor-suppressor p53 involves transcriptional modulation of the *IGF-1R* gene. p53 is a negative regulator of the cell cycle. In its hyperphosphorylated form p53 arrests cycle progression, thus preventing passage of damaged DNA to daughter cells. Wild-type p53 was shown to suppress *IGF-1R* transcription, leading to reduced IGF-1R levels and to a decrease in ligand-induced IGF-1R activation. As a net result, proliferation is reduced and apoptosis is increased (left panel). Malignant cells often include a mutant p53 gene. Mutated p53 proteins are able to transactivate the *IGF-1R* gene, leading to augmented IGF-1R levels and enhanced IGF-induced IGF-1R phosphorylation. Activation of the IGF signaling pathway is usually associated with rapid tumor growth and prevention of apoptosis (right panel). A similar paradigm was identified for other p53-family genes, including p63 and p73 (Nahor *et al*., 2005).

a truncated version of the molecule (del185AG, commonly known as the 'Ashkenazi mutation') had a reduced effect on IGF-1R expression. In terms of the mechanism of action of BRCA1, electrophoretic mobility shift assay assays using the *in vitro*-translated BRCA1 failed to reveal binding of the protein to *IGF-1R* promoter sequences (Abramovitch *et al*., 2003). However, BRCA1 was capable of binding zinc-finger protein Sp1, hence preventing this nuclear protein from transactivating the *IGF-1R* gene.

By analogy to the paradigm postulated above for p53, loss of inhibitory regulation of the *IGF-1R* gene by mutant BRCA1 may lead to enhanced IGF-1R expression (and, in turn, enhanced activation by endocrine and/or locally produced IGF-1/IGF-2). This postulate was indeed confirmed by immunohistochemical analysis of IGF-1R expression in primary breast cancer specimens derived from BRCA1 and BRCA2 mutation carriers. Quantitative analyses revealed that IGF-1R levels were higher in tumors of BRCA1 and BRCA2 mutation carriers compared with those from matched sporadic tumors (Maor *et al*., 2007b) (Figure 4). Consistent with this data, a study by Voskuil *et al*. (2004) showed that the levels of some IGF system components, including IGF-1R, in normal and tumor breast tissue were higher in individuals with a strong family history of breast cancer than in individuals without a family history. Furthermore, evidence in support of bidirectional interplay between the IGF-1 system and BRCA1 was provided by studies showing that IGF-1 increases BRCA1 expression and enhances *BRCA1* promoter activity (Maor *et al*., 2007a).

In summary, the significance of IGF-1R expression as a determinant of prognosis in breast cancer has been a controversial issue for many years (Happerfield *et al*., 1997). A recent study has shown that IGF-1R is differentially expressed with variable prognostic impact among breast cancer subtypes (Yerushalmi *et al*., 2011). As alluded to early, IGF-1R activation is of crucial importance and breast cancer patients with high levels of phosphorylated IGF-1R were reported to have reduced survival (Law *et al*., 2008). Finally, the functional and physical interactions between the IGF axis and high-penetrance breast cancer genes hold major clinical relevance. We may postulate that *BRCA* mutational status may predict responsiveness to IGF-1R-targeted therapies.

Loss-of-function mutation of VHL in kidney cancer enhances IGF-1R expression

A similar paradigm for negative regulation of the *IGF-1R* gene was provided by the von-Hippel Lindau (VHL) tumor suppressor. VHL is the substrate recognition component of an E3 ubiquitin ligase complex and it has a role in the oxygen-dependent proteolysis of the α subunits of hypoxia-inducible factors (HIFs) (Conaway and Conaway, 2002; Wiesener *et al*., 2009). At normal oxygen pressure, HIF-α subunits are hydroxylated on proline residues, targeting them for VHL-mediated ubiquitylation and proteosomal degradation. Under hypoxia conditions, the absence of oxygen-dependent hydroxylation of HIF-α prolines allows HIF-α to accumulate and translocate to the nucleus, triggering transcription of hypoxia-inducible genes.

VHL mutations occur in approximately 75% of clear cell renal cell carcinoma. Inactivation of the VHL protein allows normoxic accumulation of HIF-α subunits, leading to constitutive expression of hypoxia-inducible genes. In a recent study, IGF-1R values were unaffected by hypoxia, however, were found to be higher in clear cell renal cell carcinoma cells harboring a mutant inactive VHL than in isogenic cells expressing a wild-type VHL. Furthermore, *IGF-1R* promoter activity and mRNA levels were lower in clear cell renal cell carcinoma cells expressing a wild-type VHL, suggesting that VHL negatively controls IGF-1R expression (Yuen *et al*., 2007).

Finally, the mechanism of action of VHL in the specific context of *IGF-1R* gene regulation involves interaction with Sp1 and, therefore, resembles the mechanism described above for BRCA1 and p53. Specifically, *IGF-1R* promoter activity was suppressed by full-length VHL but only partially by a truncated VHL lacking an Sp1-binding motif. The clinical relevance of these findings was confirmed by measurements showing that IGF-1R mRNA levels were higher in clear cell renal cell carcinoma biopsies than in benign

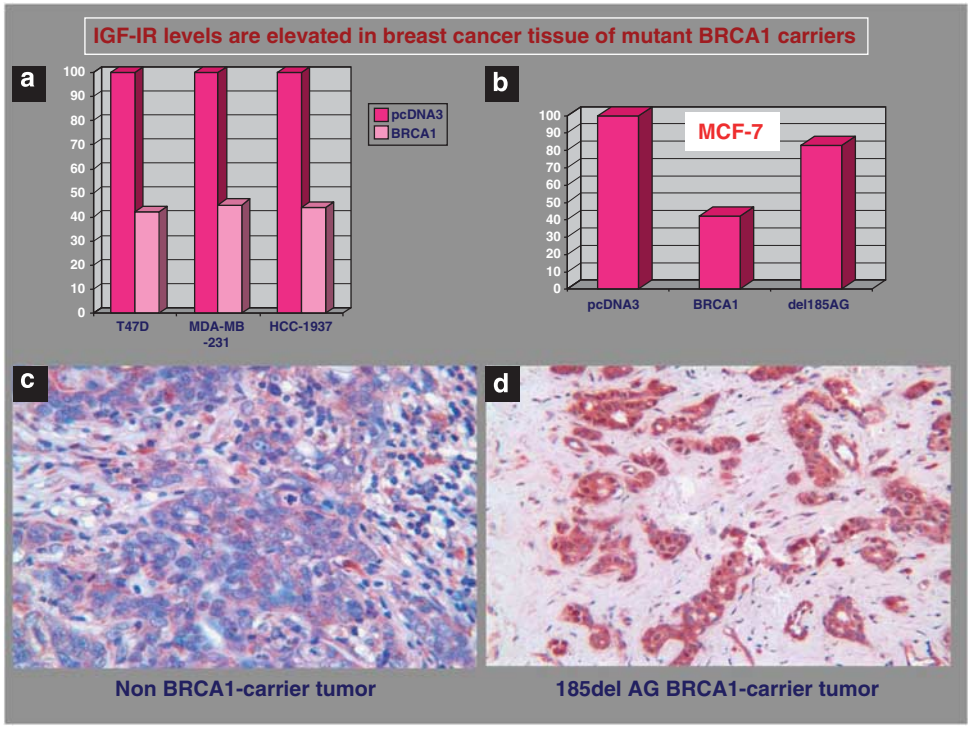

Figure 4 IGF-1R levels are elevated in breast cancer tissue of mutant BRCA1 carriers. (**a**) BRCA1 is a transcription factor whose mutation has been linked to the etiology of familial breast and ovarian cancer. BRCA1 functions as a tumor suppressor capable of arresting growth of mammary cells, whereas mutant BRCA1 is unable to halt proliferation. Consistent with its tumor-suppressor role, expression of wild-type BRCA1 in various breast cancer-derived cell lines (T47D, MDA-MB-231, HCC-1937) led to significant repression of a co-transfected *IGF-1R* promoter (Abramovitch *et al*., 2003). (**b**) The del185AG variant, which encodes a truncated BRCA1molecule, is the most common mutation in familial breast and ovarian cancer among Ashkenazi Jews. Del185AG BRCA1 was unable to repress *IGF-1R* promoter activity in MCF7 cells in co-transfection experiments. (**c**, **d**) Immunohistochemical analyses using antibodies against both the extracellular and intracellular domains of the receptor revealed that IGF-1R was expressed in all primary tumors and in surrounding normal tissues. IGF-1R immunostaining was predominantly cytoplasmic, although in several of the tumors associated with BRCA1 mutations, IGF-1R was also observed in the plasma membrane. Quantitative evaluation of IGF-1R staining revealed a higher score in BRCA1-associated tumors compared with those from non-carriers (4.64 ± 0.5 vs 2.64 ± 0.24, mean ± s.e.m., $P < 0.002$). Hence, *loss-of-function* mutation of the BRCA1 gene in familial breast cancer led to upregulation of the *IGF-1R* gene, a downstream target for BRCA1 action.

kidney. Taken together, these studies have identified a role for tumor-suppressor VHL in suppressing *IGF-1R* transcription and mRNA stability in kidney. VHL inactivation leads to IGF-1R upregulation, contributing to renal tumorigenesis.

The *IGF-1R* gene is a target for aberrant transcription factors

The hypothesis that the *IGF-1R* gene is a target for tumor-suppressor action was also tested in solid pediatric tumors. In Wilms' tumor, IGF-1R mRNA levels were higher than in normal adjacent kidney tissue (Werner *et al*., 1993). Moreover, IGF-1R expression in primary tumors was negatively correlated with the expression of Wilms' tumor-1 (WT1), a zinc-finger transcription factor whose mutation is a key event in the etiology of the disease (Huff, 2011). Consistent with its tumor-suppressor role, WT1 expression inhibited *IGF-1R* gene transcription in co-transfection experiments. In addition, stable expression of WT1 led to a reduction in endogenous IGF-1R levels, IGF-1-stimulated cellular proliferation and anchorage-independent growth (Werner *et al*., 1995). However, unlike p53, BRCA1 and VHL, whose mechanisms of action do not seem to involve direct binding to *IGF-1R* promoter sequences, WT1 displayed specific binding to consensus early growth response/WT1 elements in the proximal promoter.

An interesting example of disruption of inhibitory control of the *IGF-1R* gene in childhood tumors is provided by desmoplastic small round cell tumor. Desmoplastic small round cell tumor is an aggressive primitive tumor in children and adolescents, characterized by a recurrent chromosomal translocation, t(11;22)(p13;q12) (Gerald *et al*., 1998; Gerald and Haber, 2005). This rearrangement fuses the N-terminal (activation) domain of the Ewing sarcoma (EWS) gene, which encodes an RNA-binding protein involved in a number of cancer-related translocations, to the C-terminal, zinc-finger (DNA-binding) domain of WT1. Chimeric EWS–WT1 fusions were shown to bind early growth response/WT1 sites and to transactivate

the *IGF-1R* promoter in coexpression assays (Finkeltov *et al*., 2002; Werner *et al*., 2007). Hence, tumor-specific fusion of EWS to WT1 in desmoplastic small round cell tumor abrogates the tumor-suppressor role of WT1 and generates an oncogenic molecule capable of binding and transactivating WT1 target genes, including the *IGF-1R*.

Oncogenes 'adopt' the *IGF-1R* signaling pathway

Although classical tumor suppressors were consistently shown to control *IGF-1R* gene expression in a negative manner, as exemplified in previous sections, oncogenic agents, on the other hand, are usually associated with enhanced transcription of the *IGF-1R* gene and/or augmented cell-surface IGF-1R activation. In other words, oncogenes 'adopt' the IGF-1R signaling pathway as *their* mode of action. Certain oncogenes, for example, $pp60^{src}$, the protein encoded by the *src* oncogene of Rous sarcoma virus, were shown to stimulate the constitutive phosphorylation of the IGF-1R tyrosine kinase domain (Peterson *et al*., 1994). It has been estimated that $\sim$10–50% of the receptors are phosphorylated in the unstimulated *src*-transformed cell and that addition of IGF-1 synergistically increased the extent of receptor phosphorylation (Kozma and Weber, 1990). These results are consistent with the notion that $pp60^{src}$ alters growth regulation by rendering the cells constitutively subject to a mitogenic signal. Other oncogenes, including c-myb, can *trans*activate the *IGF-1R* promoter, with enhanced *IGF-1R* gene transcription and biosynthesis (Reiss *et al*., 1991; Travali *et al*., 1991). Similarly, the hepatitis B virus X protein enhanced IGF-1R mRNA levels in hepatocellular cancer cell lines, suggesting that hepatitis B virus X exerts its role in the etiology of this malignancy *via* transactivation of the *IGF-1R* gene (Kim *et al*., 1996). Taken together, regardless of their particular mode of action, cellular and viral oncogenes require an intact, activated IGF-1R signaling pathway in order to elicit their transforming activities. Of importance, the IGF-1R is also responsible of mediating oncogene-directed differentiative events. For example, *IGF-1R* has been identified as a target for Δ40p53, a transactivation deficient isoform of p53, which controls the switch of pluripotent embryonic stem cells to differentiated somatic cells (Ungewitter and Scrable, 2010). Specifically, Δ40p53 acts as a master regulator of this switch by modulating IGF-1R levels.

MicroRNAs regulate IGF-1R expression

In addition to classical tumor suppressors and oncogenes, recent studies have shown that the *IGF-1R* gene can be regulated by microRNAs (miRs) (Hornstein and Shomron, 2006). MiRs are small non-coding RNAs that control target gene expression at post-transcriptional levels in a sequence-specific manner (Zalts and Shomron, 2011). For instance, a bioinformatic analysis has identified the IGF-1R mRNA as a potential target for miR-7 (Jiang *et al*., 2010). Ectopic expression of miR-7 led to a significant decrease in IGF-1R mRNA and protein levels in tongue squamous carcinoma cells. Luciferase reporter assays confirmed the targeting of miR-7 to three candidate sequences in the 3′-untranslated region of the *IGF-1R* gene. In addition, miR-7-mediated downregulation of IGF-1R expression was shown to attenuate the IGF-1-stimulated activation of Akt. In view of its putative tumor-suppressor role, it was suggested that miR-7 might have an important role in tongue squamous carcinoma cell etiology *via* modulation of IGF-1R expression.

An interesting novel regulatory mechanism was recently demonstrated in EWS, linking in a complex manner the activities of the IGF-1 signaling pathway, the EWS-Fli1 oncogene, and miRs (McKinsey *et al*., 2011). Specifically, a collection of miRs with predicted targets in the IGF-1 signaling axis was shown to be repressed by EWS-Fli1, the hallmark oncoprotein of EWS. Furthermore, miRs in this group negatively regulated the expression of pro-oncogenic components of the IGF pathway, including IGF-1, IGF-1R and mammalian target of rapamycin (mTOR). This study, thus, depicts a novel oncogenic mechanism in EWS, involving post-transcriptional derepression of IGF signaling by the EWS–Fli1 fusion oncoprotein *via* miRs.

Finally, miRs control of the *IGF-1R* gene and, in a broad sense, of the entire IGF-1 signaling axis, provides an additional level of regulation, which may have important relevance in terms of metabolic control of normal and transformed cells as well as in the regulation of growth and development processes. MiRs, in addition, are attractive biomarkers in various types of cancer and might even constitute appealing therapeutic targets (Brase *et al*., 2010; Nana-Sinkam and Croce, 2011).

The role of the IGF-1R in metabolic regulation

In addition to its growth-inducing activities, IGF-1 exhibits a number of insulin-like effects. Metabolic effects of IGF-1 include, among others, elevation of glucose uptake and hypoglycemia, without lowering free fatty acid levels (Guler *et al*., 1987; Jacob *et al*., 1989). In addition, IGF-1 was shown to improve renal function by increasing renal blood flow and glomerular filtration rate (Guler *et al*., 1989). The question through which receptor (IGF-1R, IR) are those activities mediated has been the topic of controversial research. There is, however, wide consensus today that significant portions of the metabolic activities of IGF-1 are directly mediated *via* the IGF-1R (LeRoith and Yakar, 2007).

One of the best-characterized alterations that take place in transformed cells is an adjustment in adenosine triphosphate (ATP)-generating pathways, known as the *Warburg effect*. This effect represents a shift from ATP production *via* oxidative phosphorylation to ATP generation *via* glycolysis (Warburg, 1956). Glycolysis

(which can take place also under normal oxygen pressure, that is, aerobic glycolysis) is less efficient than oxidative phosphorylation in terms of ATP production and, therefore, tumor cells require a large supply of glucose. Glucose uptake is known to be strongly stimulated by insulin and IGF-1 (Werner *et al*., 1989). The phosphatidylinositol 3 kinase pathway, which is activated following IGF-1R (and other growth factor receptors) activation, is usually altered in cancer cells (Cairns *et al*., 2011). Activation of the phosphatidylinositol 3 kinase pathway provides not only antiapoptotic and mitogenic signals but has also a marked impact on cancer cell metabolism. Akt/protein kinase B, a downstream target of phosphatidylinositol 3 kinase, has been shown to stimulate the glycolytic pathway, hence favoring energy production in the tumors (Plas and Thompson, 2005). Additional signaling proteins downstream of IGF-1R with key roles in the regulation of cancer cell metabolism are mTOR and AMP-activated protein kinase (AMPK). mTOR, which is usually constitutively activated in cancer cells, directly stimulates lipid and protein synthesis and is responsible for a number of metabolic adaptations (Guertin and Sabatini, 2007). Similarly, AMPK is a critical energy sensor and its spectrum of activities is, in general, opposite to that of mTOR. AMPK functions as a metabolic checkpoint capable of modulating the cellular reaction to energetic changes (Shackelford and Shaw, 2009).

Finally, p53, which was shown previously to constitute an upstream regulator of the *IGF-1R* gene, has also an important role in the control of metabolism (Vousden and Ryan, 2009). Thus, wild-type p53 inhibits the phosphatidylinositol 3 kinase pathway *via* stimulation of phosphatase and tensin homologue deleted on chromosome 10 (PTEN). As a result, glycolysis is deactivated. It is conceivably that *loss-of-function* mutation of p53 in tumor cells may lead to stimulation of the glycolytic pathway, consistent with the Warburg theory. The role of IGF-1R as a potential mediator of this effect has not yet been established.

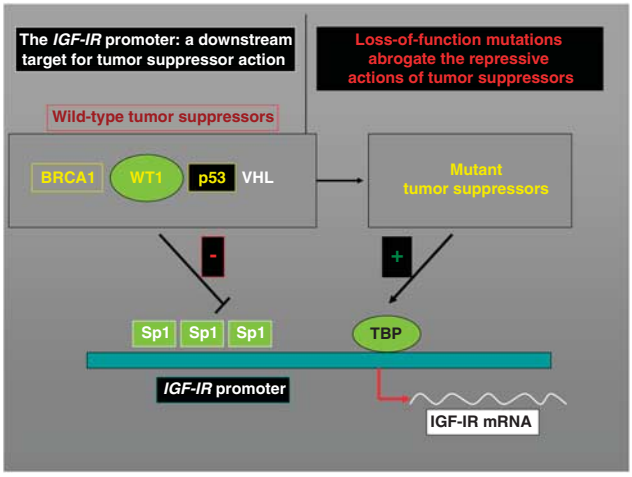

Figure 5 The *IGF-1R* promoter is a downstream target for tumor-suppressor action. The mechanism of action of multiple tumor suppressors involves transcriptional suppression of the *IGF-1R* gene. Certain tumor suppressors (for example, BRCA1, p53, VHL) elicit their inhibitory effect *via* protein–protein interactions with basal transcriptions factors, including Sp1 and TATA-binding protein (TBP). Other tumor suppressors (for example, WT1) were shown to directly bind to consensus elements in the promoter region. As a result of negative regulation of *IGF-1R* gene expression, the cell is most likely to remain at a post-mitotic stage and out of the cell cycle. The etiology of cancers associated with *loss-of-function* mutation of tumor suppressors is probably linked to the inability of these mutated proteins to suppress their downstream targets, including the *IGF-1R* gene. *Gain-of-function* mutations of oncogenes are in many cases correlated with increased *trans*activation of the *IGF-1R* promoter. Interactions between positively acting and negatively acting transcription factors are expected to determine the level of expression of the *IGF-1R* gene and to affect the proliferative status of the cell.

Conclusions

The IGFs are important players in a network of biochemical events linking metabolic and mitogenic pathways. The bioactivities of IGF-1 and IGF-2 depend on the concerted actions of a number of factors, including nutritional status, developmental stage, ligand biosynthesis, interactions with other hormonal systems, regulation of ligand bioavailability by IGFBPs and others. Deregulation of the insulin/IGF axis has major pathological implications, ranging from nutritional–hormonal–metabolic conditions to disorders of proliferation. In addition, the insulin/IGF axis has a profound effect on life span.

In this review, we provided evidence that the mechanisms of action of multiple cancer genes involve transcriptional modulation of the *IGF-1R* promoter (Figure 5). The etiology of cancers associated with *loss-of-function* mutation of tumor suppressors is, most probably, correlated with the inability of mutant tumor suppressors to suppress their downstream targets, including the *IGF-1R* gene. Similarly, *gain-of-function* mutations of oncogenes are associated with increased *trans*activation of the *IGF-1R* promoter. Constitutive activation of the IGF-1R signaling pathway is a fundamental requirement for acquisition of a neoplastic phenotype. Elucidation of the interplay between cancer genes and the IGF-1R axis will improve our ability to deliver IGF-1R-targeted therapies in a more effective manner.

Conflict of interest

The author declares no conflict of interest.

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