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Role of IGF-1 Receptor in Radiation Response[1]

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Abstract

Insulin-like growth factor 1 receptor (IGF-1R) is a transmembrane receptor tyrosine kinase involved in the development and progression of cancer whose activation strongly promotes cell growth and survival. IGF-1R exerts its main actions through the activation of the mitogen-activated protein kinase and phosphoinositide 3-kinase pathways. In addition to their traditional roles, IGF-1R activation has been associated with increased radioresistance both *in vitro* and *in vivo*, although the molecular mechanisms behind this process are still unclear. Recently, IGF-1R has been associated to new partners as major vault proteins, BCL-2, BAX, or Ku70/80, related to radiochemotherapy resistance, regulation of apoptosis, and nonhomologous end-joining DNA repair. Here, we review these novel associations of IGF-1R trying to explain the resistance to radiotherapy mediated by IGF-1R. Finally, we revised the role of new therapies leading to block the receptor to enhance the efficacy of radiation.

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Insulin-like Growth Factor 1 Receptor: A Brief Overview of Structure and Function

The insulin-like growth factor 1 receptor (IGF-1R) is a cell membrane receptor widely distributed in human tissues. Its structure and functions have been deeply explored during the last 20 years. The final structure of the IGF-1R is a homodimer with two α extracellular subunits and two β transmembrane subunits disulfide bonded [1]. The α subunit contains the ligand binding domain, whereas the β subunit is formed by a transmembrane domain, an intracellular domain with tyrosine kinase activity, and a C-terminal domain with protein interaction functions [2]. IGF-1 binds with high affinity to IGF-1R. The IGF-1R is also bound by IGF-2 and by insulin. The concentration of circulating IGF-1 is mainly dependent on production by the liver, which is tightly controlled by growth hormone (GH). The bioavailability of the ligands is controlled by a family of IGF-binding proteins (IGFBP1-6). The ratio between free and IGFBP-bound IGF is important in determining the potency of the growth factor. Once the receptor is activated by the binding of IGF-1, it triggers a signaling transduction cascade to the cell nucleus thus modulating some cellular functions through the regulation of transcription factors. The variety of cellular responses mediated by IGF-1R is the result of a combination of downstream signaling pathways. These pathways include the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, which mediate proliferation and cell survival (Figure 1).

The MAPK pathway is a wide cascade signaling that encompasses a high number of protein kinases, mainly mediating the increase of

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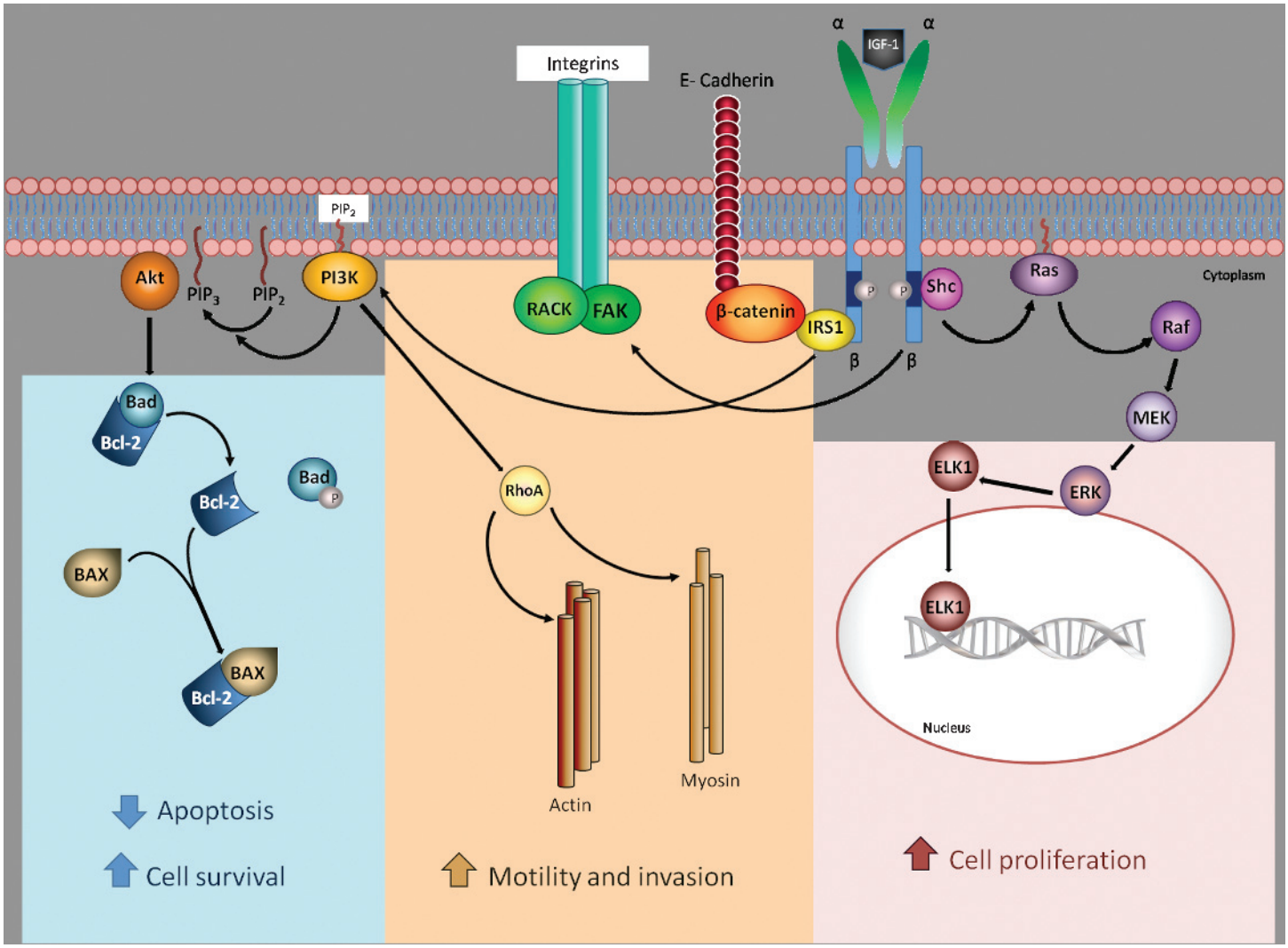

Figure 1. Signaling pathways of IGF-1R and its related functions in the cell. The figure shows the IGF-1R receptor, its main substrates, and the signaling pathways triggered as a consequence of its activation. Different colors encompass molecules implicated in the different cellular functions: blue, promotion of cell survival and antiapoptotic effect; orange, cell motility and invasion; and pink, cell proliferation and mitogenic activity. Arrows represent activation. Note that this diagram is a simplification and some effectors could be missing.

cell proliferation and differentiation. IGF-1R links with MAPK signaling through the activation of Src homology and collagen (Shc) protein by phosphorylation. The activation of these proteins is followed by a sequence of protein kinase phosphorylations through the cytoplasm (Ras [human homolog of rat sarcoma], Raf [protein serine/threonine kinase encoded by the *raf* oncogene], and MEK [MAPK kinase kinase]), which reaches the extracellular signal–regulated kinase 1 and 2 (ERK1 and ERK2) [3]. Nonetheless, this pathway also seems to control apoptosis through the proto-oncogene *Crk-II* by a Ras-dependent, Raf-1/MAPK–independent pathway [4].

The insulin receptor substrate 1 (IRS-1) is also activated when IGF-1R is phosphorylated. This protein interacts with p85 (phosphatydilinositol-3′-kinase regulatory subunit), activating the catalytic subunit (p110) of PI3K, which induces the production of activated phospholipids as a downstream signal [5]. Protein kinase B (Akt) is activated by the interaction with these phospholipids through the 3-phosphoinositide–dependent protein kinase. Akt interacts with the complex BCL-2/BAD (B-cell leukemia protein/Bcl-associated death promoter), inactivating BAD, causing the dissociation of the complex, and thus releasing the antiapoptotic BCL-2 protein mainly through the regulation of caspases [6]. BAD phosphorylation is one of the major mechanisms by which IGF-1R induces antiapoptosis and cell survival [7].

IGF-1R activation has been associated with cell adhesion, cell motility, and tumor metastasis [8]. IGF-1R activation disrupts β-catenin/E-cadherin complexes favoring cell detachment. Motility and migration are enhanced by cross talk between the IGF-1R, integrins, focal adhesion kinase, and the RACK1 scaffolding protein and by Rho-A activation, leading to actin reorganization and actin/myosin contractility [9]. IGF-1R stimulates the annexin II secretion to the extracellular matrix promoting activation of plasminogen, cell adhesion, and tumor metastasis and invasion [10,11]. It is suggested that the mechanism by which IGF-1R interacts with annexin II is independent of the main signaling pathways known of IGF-1R [12]. IGFs induce the expression of matrix metalloproteinases, required for invasion, and stimulate angiogenesis by activating endothelial nitric oxide synthase

and inducing expression of hypoxia-inducible factor-1α and vascular endothelial growth factor [9].

These cellular functions are controlled not only by IGF-1R but also by other tyrosine kinase receptors that share the same signaling pathways than IGF-1R. This is the case of the epidermal growth factor receptor (EGFR) widely related to cell transformation [13–15]. Because of their similar mechanisms of action, several publications have studied the interaction between IGF-1 and EGF receptors. The combine effect of IGF-1R and EGFR was firstly reported by Coppola et al. in 1994 [16]. In their study, it is shown that at least in mouse embryo fibroblasts, the presence of IGF-1R is required to promote mitogenic and transforming effect of the EGFR. In 2000, Roudabush et al. [17] defined a cross talk between IGF-1R and EGFR. The presence of IGF-1R seems to have a relevant role as a mechanism of resistant to anti-EGFR therapy [18]. These interactions between molecules must be taken into account when planning the most appropriate treatment.

IGF-1R in Radiotherapy

The role of IGF-1R expression in cancer has been studied in depth [19–21]. However, the implication of this protein in radiation response remains unclear. Radiation causes the loss of structure and function of most biologic molecules, including DNA. This loss of structure of the DNA molecule includes nucleotide excision, single-strand breaks (SSBs) and double-strand breaks (DSBs). The cells have developed, along the evolution, some mechanisms to repair DNA damages and thus increase their chance of survival; however, cells start the process of apoptosis if the DNA damages are not repaired. Radiation promotes a cellular state in which multiple DNA damages are produced. The most important radiation-induced DNA damage is the DSB [22]. This state is lethal to the cell if it is kept unrepaired. However, cells can reverse this damage by two different pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ) [23]. The NHEJ operates throughout the cell cycle, and it is thought to be the most extensive DSB repair pathway in mammalian cells [24]. Both HR and NHEJ lead to an increase in cell survival, but in the second case, the nonspecific recombination usually results in a loss of information of the damaged cells, favoring the survival of altered phenotypes with genetic instability. Under these conditions, cells could avoid the apoptosis (i.e., mediated by IGF-1R overexpression or altered p53), which could favor tumor malignancy and reduced tumor response to treatments [22].

The prognostic significance of IGF-1R in the response of cancer cells to radiation has been evaluated in multiple studies. In mouse embryo fibroblast, those cells without IGF-1R expression had higher radiation-induced apoptosis, whereas the cells overexpressing IGF-1R had this process inhibited [25]. Similar results have been reported in other cell lines where overexpression of IGF-1R induces radioresistance [26]. Interestingly, physiological levels of IGF-1R are an obligatory requirement for the establishment and maintenance of the transformed phenotype [27]. In estrogen receptor (ER)–positive breast cancer cell lines, the levels of the IGF-1R and IRS-1 are often elevated, and these characteristics have been linked to increased radioresistance and cancer recurrence [28]. In mouse melanoma cells, where IGF-1R expression was artificially downregulated, the radiosensitivity was higher compared with control cells [29]. These *in vitro* results highlight the usefulness of IGF-1R in the clinical prognosis of cancer patients treated with radiotherapy (Table 1). The first *in vivo* study on this field was published in 1997 by Turner et al. [26]. They hypothesized that those tumors that had recurred in the irradiated breast after lumpectomy would represent an especially radioresistant subgroup. The IGF-1R protein expression levels were examined by immunohistochemical technique in tumor specimens from local relapse after radiation treatment of patients compared with matched nonrelapse control cases. The staining IGF-1R was rated on a 4-point scale: 0, none; 1+, light; 2+, moderate; 3+, heavy; and 4+, intense. A value of 2+ intensity was considered positive. IGF-1R was overexpressed in those cases with disease recurrence after radiation therapy [26]. This first clinical finding was in line with previous work, which demonstrated the antiapoptotic action of this oncoprotein [30,31]. However, it is not yet clear whether the IGF-1R–mediated effect on radiation response involves any of the known apoptotic regulators, such as the BCL-2 family member proteins or the CPP32 serine-cysteine proteases or if it is mediated by the interaction with other molecules. In 2007, Lloret et al. [32] studied the protein expression of IGF-1R in cervical cancer patients treated with chemoradiotherapy. Tissue specimens were collected at diagnosis. IGF-1R staining, observed in cell membrane, was semiquantitatively scored from negative (–) to slightly (+), moderately (++), and strongly positive (+++), considering IGF-1R overexpression of those tumors showing moderately/strongly positive staining. IGF-1R was expressed in 56 (93.7%) of 60 patients, and no relation was found with clinicopathologic variables. Complete response after treatment (the most important prognostic factor for survival) was observed in 50 patients. Thus, they analyzed the role of IGF-1R on long-term local control

Table 1. Published Studies Associating IGF-1R Expression and Clinical Outcome.

Reference	No. Patients	Type of Tumor	Treatment	Absolute Expression (%)	Relative Expression	LRDFS	DMFS	DFS	OS
Turner et al., 1997 [26]	47	Breast cancer	Lumpectomy and RT	43.0	High	Worse (P = .004)	—	—	—
Lloret et al., 2007 [32]	60	Cervical carcinoma	RT, BT and ChT	80.0	High	Worse (P = .045)	ns	Worse (P = .045)	Worse (P = .032)
Lloret et al., 2008 [51]	60	Cervical carcinoma	RT, BT and ChT	80.0	High*	Worse (P = .006)	Worse (P = .050)	Worse (P = .006)	Worse (P = .007)
Yuan et al., 2008 [33]	75	NPC	Not reported	56.0	High	Worse (P = .005)	—	—	Worse (P = .005)
Peiró et al., 2009 [36]	197	Breast cancer	CX and RT	51.5	High	Better (P = .004)	—	—	—
Taunk et al., 2010 [34]	345	Breast cancer	CX and RT	57.0	High	ns	ns	—	Worse (P = .022)†
Lara et al., 2011 [35]	131	Oral cavity carcinoma	CX and RT	45.8	High	Worse (P = .016)	ns	Worse (P = .029)	Worse (P = .009)
Henríquez-Hernández et al., 2011 [53]	60	Cervical carcinoma	RT, BT and ChT	80.0	High‡	Worse (P < .0001)	Worse (P = .010)	Worse (P < .0001)	Worse (P < .0001)

BT indicates brachytherapy; ChT, chemotherapy; CX, surgery; DMFS, distant metastasis-free survival; DFS, disease-free survival; LRDFS, locoregional recurrence-free survival; ns, not significant; NPC, nasopharyngeal carcinoma; OS, overall survival; RT, radiotherapy.

*Combined with MVP expression.

†Only in node-negative patients.

‡Combined with MVP/BCL-2 expression.

and survival in complete responder patients. All of the 19 patients with low IGF-1R tumors were free of local, distant, or death-related disease *versus* 6 of the 31 patients bearing high IGF-1R tumors, concluding that overexpression of IGF-1R correlated to a worse long-term local control of the disease in patients who initially responded to the treatment [32]. Yuan et al. [33] reported that IGF-1R expression may play an important role in the invasion, metastasis, and recurrence in patients with nasopharyngeal carcinoma. Tissue specimens were collected during biopsy, before treatment. A semiquantitative scoring scheme was applied, considering both the extent and the intensity of staining: 0, completely negative; 1+, less than 25% positive tumor cells; 2+, 25% to 50% positive tumor cells; and 3+, more than 50% positive tumor cells. The staining intensity was expressed as 0 to 3, denoting absent, weak, moderate, or strong reactivity. The final immunoreactive score was the product of multiplying these two parameters. An immunoreactive score of 4 or higher was considered as overexpression. IGF-1R overexpression was detected in 42 (56%) of 75 tissues. Together with EGFR, IGF-1R had increased expression in tumors with lymph node metastases, and there was a positive correlation between protein positivity for EGFR/IGF-1R and recurrence. There was a significantly higher 5-year survival rate in the EGFR/IGF-1R protein–negative groups compared with the EGFR/IGF-1R protein–positive groups [33]. Recently, Taunk et al. [36] analyzed the prognostic significance of IGF-1R in a cohort of early-stage breast cancer patients treated with breast-conserving surgery and radiation therapy, trying to determine whether overexpression of IGF-1R correlated to long-term outcomes, particularly local control. Tissue specimens were collected at surgery, before chemoradiation therapy. The intensity of IGF-1R staining was scored as 0 (0% of cells immunoreactive), 1+ (1%-9% of cells immunoreactive), 2+ (10-40% of cells immunoreactive), or 3+ (>50% of cells immunoreactive). Cases scored with 0 and 1 were considered as a group with negative expression levels, whereas cases scored with 2 and 3 with more than 10% of cells staining were considered as positive. Of the 345 evaluable cases, IGF-1R overexpression was noted in 197 cases (57%). IGF-1R expression did not predict survival in the whole series. Nonetheless, on the subset analysis of node-negative patients (good prognosis), tumors overexpressing IGF-1R had a significant reduction in overall survival but no apparent effect on local control [34]. Similar results have been recently reported in oral cavity squamous cell carcinoma patients [35]. Tissue specimens were collected at diagnosis. IGF-1R staining was semiquantitatively scored considering IGF-1R overexpression those tumors showing moderately/strongly positive staining. Of 131 patients, 101 (77.1%) expressed IGF-1R. IGF-1R overexpression was predictive of poor clinical outcome (including local disease-free survival, disease-free survival, and cause-specific survival) in patients experiencing advanced stages of the disease (III-IV) who were referred to postoperative radiotherapy [35]. Only one study has reported contradictory results [36]. Tissue specimens were collected at surgery, before chemoradiation therapy. The IGF-1R staining was scored semiquantitatively according to the percentage of positive tumor cells and intensity (from 0 to 3+; score, 0-300). High levels of active IGF-1R were related to better response to treatment (lumpectomy and radiotherapy) in early breast cancer patients negative for node, thus considering low active IGF-1R as a bad prognosis factor for local recurrence [36]. The discrepancies may be due to the selection of patients (incompletely described series or differences in the treatment protocols or in the analysis strategy); nevertheless, the immunohistochemistry assay results may not be comparable because different antibodies and scoring system were applied. Finally, the gene expression level of IGF-1R was studied by Hirano et al. [37] in 46 endometrial, 32 cervical, and 20 ovarian cancers and in 28 normal endometrium, trying to disclose the molecular mechanisms behind the apparent clinical association observed between IGF-1R overexpression and radiation response. The authors agreed with the clinical results, reporting overexpression of IGF-1R in cervical tumors compared with normal tissues [37].

Molecular Pathways Involved in IGF-1R–Mediated Radiation Resistance

Although the association between IGF-1R expression and radiation response seems to exist, little is known about the mechanisms behind this association. This fact could be explained in a classic way through the involvement of IGF-1R in modulating cell proliferation or in an alternative way through the relation of IGF-1R in modulating DNA repair in association with other partners such as ataxia telangiectasia mutated (ATM) or novel molecules such as major vault protein (MVP). Nonetheless, both pathways probably participate in the modulation of radiation response mediated by IGF-1R.

IGF-1R and Cell Proliferation

IGF-1R modulates cell proliferation through the classic and well-known interaction with the MAPK pathway [3]. Cell proliferation could be established by immunostaining of Ki67 protein. Ki67 is a nuclear protein that is expressed in cycling cells; thus, it could represent a measurement of the tumor growth fraction [38] and, consequently, could estimate tumor repopulation. The Ki67 index has been evaluated in cancer patients treated with radiotherapy, looking for a predictive assay for radiation response and prognosis. Response to radiotherapy is determined mainly by tumor proliferation, radiosensitivity, and hypoxia. During conventional radiotherapy schedules (for 5 weeks), a significant part of the daily dose per fraction is used to control cells repopulated from the previous day [39,40], decreasing tumor control probability. If tumors repopulate rapidly, this fraction will be even higher, reducing the chance of durable local control and cure. Thus, high proliferative tumors will have poor prognosis as has been shown previously in the head and neck [40–42] or bladder [43] cancer patients. In slowly proliferating tumors, repopulation is not a major concern, and high doses of radiation can be administered for longer periods, using several small fractions per day [44]. The direct relation established between IGF-1R and cell proliferation points this protein as relevant in the modulation of radiation resistance.

IGF-1R and DNA Repair and Apoptosis

X-rays interact with molecules of body tissue, causing ionization and release of electrons, which cause secondary damage to adjacent tissues including DNA through an oxygen-dependent mechanism. The resultant damage can be either SSBs or DSBs within DNA. DNA lesions are recognized by DNA damage cell cycle checkpoints that lead to repair pathways. The *ATM* gene reveals that it encodes a protein called ATM kinase, which is a member of the PI3K, a pathway that plays a critical role in cell progression by promoting cell proliferation and inhibiting apoptosis [45]. It has been identified that ATM kinase is a central component for DNA damage checkpoint pathway [46]. On exposure to ionizing radiation, ATM kinase is immediately activated resulting in phosphorylation of a number of critical agents that are involved in DNA repair, apoptosis, and cell cycle arrest. Peretz et al. [47] reported a fundamental link between ATM function and IGF-1R expression and suggested that reduced expression of IGF-1R contributes to the radiosensitivity of ataxia

telangiectasia (AT) cells. AT cells expressed low levels of IGF-1R and were more radiosensitive than cells from apparently healthy individuals. Complementation of AT cells with ATM complementary DNA results in increased IGF-1R promoter activity and IGF-1R levels, which, in turn, led to increased radioresistance. Molecular studies suggest that IGF-1R signaling can modulate the function of ATM and support the concept of targeted IGF-1R down-regulation as a potential treatment of radioresistant tumors [29].

Vaults are ribonucleoprotein particles with a hollow barrel-like structure [48] and a mass of 13 MD. In mammals, it is composed of three proteins: MVP (104 kDa), the vault poly(adenosine diphosphate-ribose) polymerase (VPARP; 193 kDa), and telomerase-associated protein 1 (240 kDa), together with small untranslated RNA (vRNA) of 141 bases. MVP interacts with factors involved in apoptosis and cell proliferation, inhibiting phosphatase and tensin homolog by increasing PI3K/Akt–mediated inhibition of apoptosis through inhibition of the proapoptotic BCL-2–associated agonist of cell death (BAD) protein [49]. VPARP are proteins of the same family of poly-(ADP-ribose) polymerase 1 (PARP-1), which have an important role in controlling DNA repair and maintenance of DNA integrity [50]. An association between IGF-1R and MVP has been reported [51]. Tumors showing high MVP expression levels also had IGF-1R overexpression. MVP and IGF-1R expression were related in clinical cervical tumors and confer reduced long-term local control in patients treated with radiochemotherapy, suggesting for the first time a strong correlation between these two proteins [51]. The interaction between IGF-1R and MVP is of great interest and can help explain the predictive role of these oncoproteins in response to radiotherapy. In a study performed on 116 women with localized cervical carcinoma, Lloret et al. [52] reported an inverse association between MVP and BAX, IGF-1R and BAX, as well as a direct association between IGF-1R and BCL-2, suggesting that IGF-1R could cooperate with MVP in preventing apoptosis by up-regulation of BCL-2 and down-regulation of BAX. The role of BCL-2 in this scenario seems to be complex and closely related to IGF-1R. This association has been recently explored by Henríquez-Hernández et al. [53] in cervical carcinoma patients. Tumors overexpressing IGF-1R showed increased levels of BCL-2 and MVP expression. BCL-2 seemed to be an important factor in the prognosis of patients, selecting a group of patients with excellent survival rates. Thus, all those cervical cancer patients treated with radiochemotherapy who presented low expression levels of MVP and/or IGF-1R and/or BCL-2 were alive after more than 10 years of follow-up. An overexpression of the three proteins is needed to develop a "bad prognosis" phenotype. Similar results were observed for local relapse disease-free survival, distant metastasis-free survival, and disease-free survival [53].

MVP is associated with the NHEJ through the interaction with Ku70/80 [52]. The Ku70/80 heterodimer binds to the broken ends of the DNA strands and joins other proteins to constitute the repair complex. It also has a strong regulatory effect on the BCL-2/BAX interaction. The Ku70/80 protein has been proposed as a key modulator of the apoptosis function [54,55]. Lloret et al. [52] showed that low levels of Ku70/80 expression were inversely related to BCL-2 expression and tumor proliferation and were directly related to BAX and altered p53 expression. Ku70/80 activity is suppressed by PARP-1 (part of vault complex), promoting HR over NHEJ [56]. The relationship between Ku70/80 and vault proteins has been recently corroborated at the clinical level [52]. MVP and Ku70/80 expression were inversely related, suggesting that MVP may have a role in favoring increased genetic instability by reducing DNA damage repair by means of NHEJ and downregulating Ku70/80 expression. Tumor progression and resistance to chemotherapy and radiotherapy also may be activated through the suppression of BAX and up-regulation of IGF-1R, resulting in increased proliferation and reduced apoptosis caused by up-regulation of BCL-2 and overexpression of altered p53 [52]. The inhibition of the IGF-1R signaling induces radiosensitivity through several pathways. Impaired IGF-1R function increased radiosensitivity by a mechanism involving the decrease in Ku-DNA binding activity and nuclear Ku86 down-regulation. This mechanism was considered p38 kinase dependent [57]. At the clinical level, Henríquez-Hernández et al. [53] have recently reported, in 50 cervical cancer patients who achieved complete response to radiochemotherapy, that Ku70/80 was significantly repressed in tumors overexpressing IGF-1R/MVP/BCL-2. Ku seems to be a central regulator of apoptosis by interacting with BAX [54] and BCL-2, which, in turn, has been shown to suppress Ku70/80, thus inhibiting NHEJ repair [55]. IGF-1R may be involved in this process through the phosphorylation of BAD, which, in turn, promotes the liberation of BCL-2 or by the association with MVP and the subsequent modulation of Ku (Figure 2). Although the prognostic significance of IGF-1R is not entirely clear, it seems that this oncoprotein could be a good prognostic factor in patients treated with radiotherapy and may be a direct target to improve the radiation response of cancer patients. IGF-1R may affect NHEJ by regulation of Ku70/80 through the interaction with MVP. The cellular mechanisms by which IGF-1R could affect Ku70/80 through MVP are unknown, and this theoretical association may be considered as a new hypothesis that could improve the knowledge about the role of IGF-1R in radiation resistance (Figure 3).

IGF-1R Targeting in Radiotherapy Treatment

The influence of IGF-1R expression in cellular functions associated to cell transformation and cancer development has been evaluated in

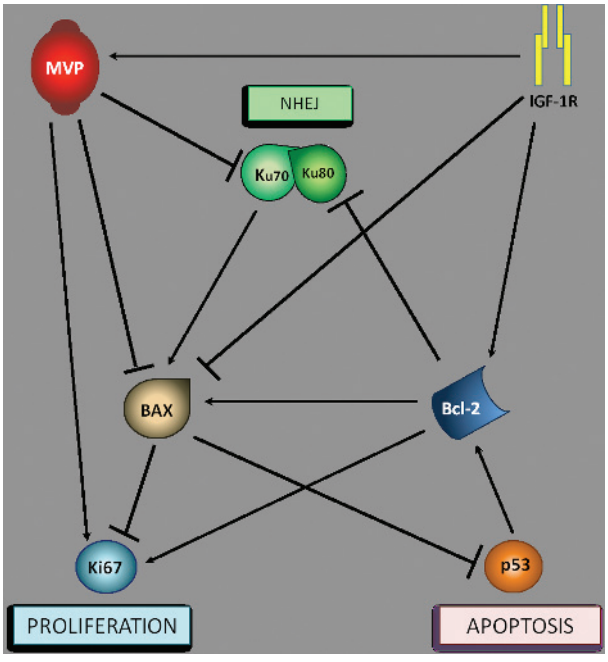

Figure 2. IGF-1R and its clinical relation to molecules involved in cell proliferation, apoptosis, and DNA repair. (→) represents positive correlation, whereas (⊣) represents negative correlation [51,52].

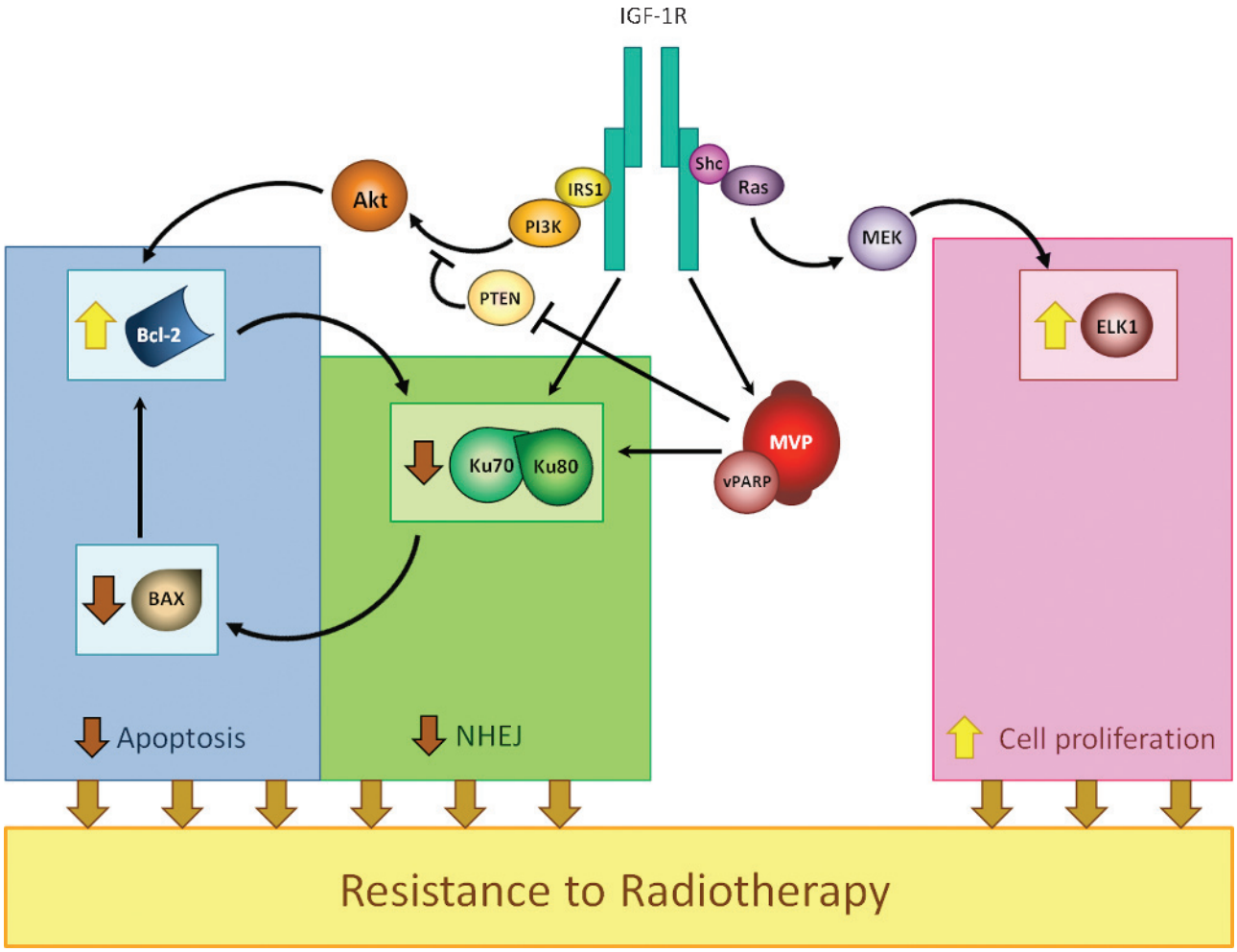

Figure 3. Main signaling pathways triggering by IGF-1R leading to RT resistance.

multiple studies, mainly focused on cancer cell lines. Most of the studies tried to explore the effect of IGF-1R inhibition to elucidate the possible use of IGF-1R as a target in cancer treatment [58]. At the same time, another line of research that leads to evaluate the implications of IGF-1R in radiation resistance was developed, trying to develop possible strategies to enhance radiosensitivity. In 2005, Cosaceanu et al. [59] found that, in non–small cell lung cancer (NSCLC) cell lines, the blockade of IGF-1R in combination with radiation had a synergistic effect on cell death and was associated with an accumulation of cells in the G_2 phase. The effect of IGF-1R inhibition has been estimated in several studies, but few of them have related this effect with radiation.

The mechanisms by which IGF-1R is inhibited are multiple and are mainly based in its binding capacity. The use of inhibiting molecules, analogous to IGF-1R ligands (IGF-1, IGF-2), is widespread, and their effects are under study [60–64]. However, this strategy has the problem of being nonspecific because these molecules can bind to homologous receptors such as the insulin receptor (IR). The use of anti–IGF-1R monoclonal antibodies has solved part of this issue. The effect of the interaction between this potential therapy and radiation response has been proven. Combined treatment with the anti–IGF-1R monoclonal antibody A12 (ImClone Systems, Inc, New York, NY) and radiation shows that the use of both treatments enhances antitumor efficacy compared with each treatment alone [65]. The same anti–IGF-1R antibody (A12) was used in head and neck cancer cell lines. The combination of this treatment with radiation induced apoptosis and increased necrosis [66]. Another anti–IGF-1R antibody, CP 751,871, was evaluated *in vitro* and *in vivo* in NSCLC cell lines. The analysis revealed that the use of CP 751,871 increased the sensitivity of NSCLC to radiation *in vitro*, increased the number of apoptotic cells, and inhibited the repair of radiation-induced DNA double-strand breaks, confirming the sensitizing effect of CP 751,871 in NSCLC to radiation [67].

The use of small interfering RNA (siRNA) to knock down the endogenous regulation of IGF-1R is also under study. The lentivirus-related siRNA IGF-1R inhibition in osteosarcoma cell lines reduced the expression of IGF-1R, and consequently, their growth rates and invasiveness increased the apoptosis and enhanced their radiosensitivity [68]. The same results were achieved in colon cancer cells [69]. Transfection of the mammalian expression vector containing IGF-1R siRNA was shown to reduce IGF-1R mRNA levels by up to 95%, reporting that combining IGF-1R siRNA and radiation significantly enhances antitumor efficacy compared with either modality alone [69].

Dual inhibition of tyrosine kinase receptors has emerged as a method to improve the efficacy of targeted therapy. IGF-1R inhibition, in combination with the inhibition of other receptors such as the platelet-derived growth factor receptor, increases cell death in high-grade glioma cell line and induces radiosensitization of those

more radioresistant cells [70]. More relevant is the association established between the EGFR and IGF-1R. The EGFR and IGF-1R pathways can communicate on multiple levels [71] and are logical targets for molecular therapy for cancer based on their frequent overexpression and established roles in the pathogenesis and progression of numerous cancers. Treatment with either A12 (anti–IGF-1R monoclonal antibody) or cetuximab (anti-EGFR monoclonal antibody) alone resulted in statistically significant inhibition of tumor-associated angiogenesis, whereas the combination treatment with A12 and cetuximab resulted in an additional inhibition of angiogenesis [72]. These findings have been observed in other studies that have demonstrated reduction of angiogenesis resulting from inhibition of IGF-1R, EGFR, or both receptors simultaneously [73,74].

Conclusions

IGF-1R is a membrane receptor that controls several cell functions such as proliferation, differentiation, cell survival (by inhibiting apoptosis), and cell adhesion. Overexpression of IGF-1R would make cells enter in a state of alteration that ends in cancer transformation. IGF-1R has been related to radioresistance not only in cell lines but also in the clinical setting, making IGF-1R expression a suitable predictive factor for radiotherapy outcomes. The mechanisms by which IGF-1R induces radioresistance could be related not only to increasing proliferation and reduced apoptosis but also to DNA repair alteration through its interaction with MVP and, indirectly, to Ku70/80 (Figure 3). These facts suggest that IGF-1R could be a good target for combination treatments with other tyrosine kinase inhibitors, chemotherapy, or radiotherapy [74–78].

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