

The importance of the IGF-signaling receptors, IGF1R and IR, in cancers and the possible ways to interrupt the pathways

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Jacobien Boiten, s1645412

Abstract:

Insulin-like growth factors (IGFs) and insulin are main regulators of cell growth in human and in animals. IGFs can bind to the IGF-receptors (IGF1R and IGF2R) or to the insulin receptor (IR). Insulin can only bind to the IR. Whereas there is only one type of IGF1R and IGF2R, two isoforms of IR exists, IR-A and IR-B. The IGFs- and Insulin receptors contains both two dimers with an extracellular alfa- and two intracellular beta domains. The IGF1R and IR can also form heterodimers with each other, the so called hybrids.

IGFs and their signal transducing receptors are thought to have an important role in carcinogenesis and in the resistance of cancers against chemotherapy. There is evidence that cancers up-regulate the expression of IGF-I and II. Additional data show that cells survive and escape from chemotherapy when IGF-I is added. Mainly, the IR-A plays a keyrole in IGF signaling. IR-A and also the hybrid form with IGF1R (HR-A) are over-expressed in neoplastic tissue. This last form especially has to be blocked because it facilitates more binding sites for IGFs, by binding IGF-I and II and insulin all with high affinity. Discussed in this paper are the ways to block IR-A and HR-A, by making a comparison to the methods used by blocking the IGF1R. The major inhibitors that are hopeful in IR-A and HR-A blocking are the tyrosine kinase inhibitors, antibodies or IGF binding proteins (IGFBPs).

There are no equal recommendations for the blockade of the IR-A/HR-A in all cancers as a result of different characteristics of every cancer. Promising IR-blocking ways for two of the most occurring cancers, breast and lung cancer, are elucidated. The use of a specific small molecule inhibitor INSM18, might have beneficial effects in the treatment of breast cancer, because this tyrosine inhibitor has also activity against the human epidermal growth factor receptor (HER2). Known is that HER2 often is up-regulated in breast cancer. For lung cancer there is evidence that a specific antibody (mAb) CP751,871 (against IGF1R) in combination with chemotherapy causes higher response rates. It might be important that also the IR-A and HR-A are targeted. This could be done by addition of small molecule inhibitors in low concentrations and antibodies. Nowadays, mAbs against IGF1R are used, but also mAbs against IR-A must be developed. Low concentrations of tyrosine kinase inhibitors must be used so IR-B is not blocked totally (because all receptors IGF1R, IR-A, IR-B, HR-A, HR-B are then blocked) but the levels are high enough to make the blocking of IR-A, IGF1R and HRA more sensitive.

Introduction

Two important receptors in insulin-like growth factor (IGF) signaling are the IGF1-receptor (IGF1R) and the insulin receptor (IR). Both signal transducing receptors bind IGFs but with a different affinity. Next to that, IGFs can bind a non-signal transducing IGF2 –receptor (IGF2R). Additionally, insulin can only bind to the IR.

Similarities are visible in structure and downstream signaling between the two IGF signal transducing (IGF1R and IR) receptors. Namely, both receptors consist of two halves, which form a homodimer receptor or a heterodimer/ hybrid receptor (a combination of the IGF1R and IR). Whereas there is only one type IGF1R, two types of IRs, IR-A and IR-B exist. IR-A binds not only insulin but also IGF-II with high affinity. The hybrid receptor of IGF1R and IR-A (HR-A) can bind all three hormones, insulin, IGF-I and IGF-II, with high affinity and therefore it is an important receptor in signaling (see also figure 1). IGF2R is an exception, beside its inability to transduce signals, this receptor can not create a hybrid. Its role is to catch the IGF-II ligand and prevent IGF-II binding to IGF-1R. (Pollak 2008, Dziadziuszko et al 2008).

Both IGF-1R and IR contain an alfa chain (extracellular domain which is binding to the ligand) and an intracellular beta-chain (including the tyrosine kinase domain, which is responsible for the intracellular signaling). When insulin or IGF bind to a signal transducing receptor autophosphorylation of the serine/threonine site occurs. Consecutive, activation of the intrinsic tyrosin kinase is activated, allowing phosphorylation of the suitable substrate, the insulin receptor substrate (IRS) and a cascade of signals is started. The PI3K-Akt and the ras-raf-MEK-ERK pathways are activated, which are both pro-survival signals. PTEN (phosphatase and tensin homolog) can inhibit the activation of the PI3K-Akt pathway (see figure 1). Other inhibitors of this pathway are AMPK and Rapamycin. (Pollak 2008 review, Dziadziuszko 2008, Sacco 2009, Keyhanfar 2007, De Meyts 2004)

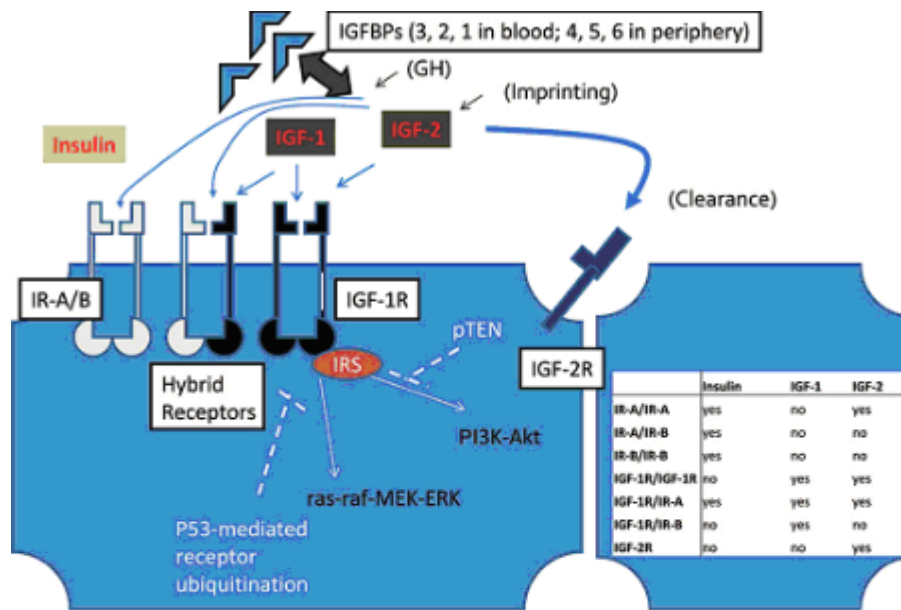


FIGURE 1. Schematic representation of the IGF axis at the level of a peripheral tissue. Three different ligands (insulin, IGF-1, IGF-2) affected by upstream elements of hormonal control (predominantly IGF-1) and imprinting (IGF-2), six different binding proteins, three different receptors, and a range of intracellular substrates and docking proteins influence intracellular signaling. The avidity of the different ligands for the different receptor combinations are shown in the embedded table. Activity in the pathway is also influenced by pTEN inhibition of the PI3K pathway, and p53-mediated receptor down-regulation, and therefore mutations in either/both genes may influence a tumor's dependence on the pathway

Figure 1. Signaling pathway of IGFs and Insulin and receptor binding possibilities (Dziedziszko et al.2008)

Although there is a difference in major action, both insulin and IGFs can induce protein translation and cause proliferation and therefore they are main regulators of cell growth in humans and animals. (Pollak, 2008). Frequently, IGFs are up-regulated in cancers. It is thought that IGFs stimulate maintenance of proliferation and therefore it has an important role in tumor genesis. Mice with an IGF-I deficiency had reduced (autologous) tumor growth and metastasis. Equally, mitogenic responses were visible in cancer cell lines when IGFs were added. Hizuka et al (1998) found that a higher molecular form of IGF-II, so called big-IGF-II, was produced in NICTH (Non-Islet Cell Tumor Hypoglycemia). They did not find big IGF-II in all patients but most of them it was determined (31 of the 44 patients had big IGF-II). Additionally, they found that serum IGF-II/IGF-I ratios in the patients with big IGF-II were also higher than the levels in the patients without big IGF-II. Fuduka discovered years later (2006) that IGF-II bound binary to IGF-binding proteins (IGFBPs) instead of forming a normal ternary complex with IGFBP-3 and Acid labile subunit (ALS). Consequently, this smaller binary complex can cross the capillary barrier and can reach the target tissues. Noted must be that both studies only tested NICTH. (Pollak 2008, Pollak 2008 review, Weroha et al. 2008, Sacco 2009, Fuduka 2006, Hizuka 1998 and Jones, 2007).

It is thought that the mitogenic response of IGFs a property of the IGF1R are, so most investigations have been done with regard to signaling via this receptor. As a matter of fact, IGF1R itself is found over-expressed on neoplastic tissue.

Additionally, Jones et al (2007) described an increase in formation of tumors with transgenic expression of IGF1R in mice. However, the IR has never been a major subject for investigation. Though, especially IR-A and HR-A might be important, because it uses the same signaling pathway as IGF1R and can also bind IGF-I and II. IR-A, as homodimer or as hybrid (HR-A), is indeed also found on neoplastic cells. Besides, gene expression databases are also showing that IR is expressed in cancers in a same amount as seen in classical insulin sensitive tissues.

Anti-tumor therapies which rely on activation of apoptosis might be influenced by IGF signaling. Namely, IGF causes a shift in the balance of pro-survival and pro-apoptotic proteins towards pro-survival. Most of the therapies are based on this principle of activation of apoptosis (for example cytotoxic chemotherapy, hormonal- and radiation therapy) and for that IGF-blocking therapies might make the existing therapies more effective. (Werhoha 2008).

Various levels of available IGFs and different sensitivity to these ligands are due to various levels of expression of the receptor genes and/or to a complex family of IGF-BPs. There is evidence that some factors (like p53, retinoids, vitamin D) regulate the increase of IGF-BP expression. Additionally, these factors regulate the decrease of ligand availability and decrease of receptor activation. Also, the growth hormone (GH) regulate concentrations of IGF-I in the blood. Insulin regulation is more complex because more regulators are involved and because the ligand also has an important role in metabolism in classic insulin sensitive tissue. Because of this complexity and because there is evidence that IGFs has strong proliferative signals by binding on IR (and also on HR), although they have low affinity for this receptor, is why we focus on the IGFs. (Pollak 2008, Pollak 2008 review, Sacco 2009).

In this paper, the role of IGF1R and IR in IGF signaling and the ways to block these receptors are investigated. These subjects are discussed compared to the information available of the IGF1R and the used methods to block this receptor.

Comparison of IR and IGF1R

Structure and signaling pathways of the IR has to be discussed and the function of different isoforms have to be clarified to investigate the role of the IR. Additionally, blocking methods for IGF1R will be reviewed and opportunities for the use of similar methods for IR blocking are discussed.

In blood plasma, IGF-II is the predominant form of circulating IGF, with plasma levels 3-7 fold higher compared with IGF-I. Data show (described by Pollak 2008) that IGF-II (and also IGF-I) is over-expressed in cancers, for example in colorectal mucosa. Normally, the maternal allele of this predominant IGF form is imprinted, which means that *IGF2* is not transcribed from the maternal allele but only from the paternal one. Loss of imprinting could cause higher levels of IGF-II in cancers because both alleles then are transcribed. The study of Rikhof (2008) describes that this up-regulation is associated with changes in histone acetylation and with methylation in the *IGF2* promotor. Next to that, increase in methylation in the *IGF2* imprinting control area was found. (Dziadziuszko 2008 and Weroha 2008, Rikhof 2008 and Wise 2006, Pollak 2008).

IGF2R is not earlier discussed extensively. There is only one type of IGF2R. This receptor can bind IGF-II but not transduce a signal because the receptor is not attached to a signal transducing pathway. Therefore, this receptor functions as a scavenger of IGF by binding to the ligand and destroy it and thereby reducing IGF binding to IGF1R. In another way, *IGF2R* could be seen as a tumor suppressor gene. Remarkably, IGF2R expression is often decreased in cancers. Additionally, forced over-expression of IGF2R (in transgenic IGF2R mice) leads to reduction in tumour growth. In contrast, changes in intensifying of the *IGF1R* are not visible common. Though, *IGF1R* amplification was determined in a subset of gastrointestinal stromal tumor cells GISTs. IR is overexpressed in neoplastic tissue. This will be discussed later. (Pollak 2008, Weroha 2008, Rikhof 2008, Wise 2006). The IGF1R and IR are both transmembrane receptors and contain large homology. Yet, there are molecular differences. The first three domains of the N-terminus (L1-CR-L2) of the IR are structurally different from the IGF1R. Mainly, this involves the ligand specific regions. Additionally, it is discovered that ligand specificity is due to site specific mutagenesis. *F39* is determined as the most important gene. Another difference between the IGF1R and IR is that IGF1R has 3 tyrosine residues in the (C-terminus of the) beta-domain and IR has only 2. (Lou 2006)

Before mentioned, some differences in IGF1R and IR structure and downstream signaling are discussed. However, more differences are discovered. There exist only one type of IGF1R, but IR has two isoforms: IR-A and IR-B. The two different isotypes of the IR are defined by alternative splicing of exon 11 in the carboxy-terminus of the extracellular alpha-domain. IR-A lacks exon 11, whereas IR-B contains this exon and has therefore a 12 amino acid extension. The isoforms are slightly different in their affinity for insulin. IR-A causes proliferation, survival and migration after binding IGF-II or insulin (using the same pathway as IGF1R). This occurs because IR-A exhibits affinity for both insulin and IGF-II though it is higher for insulin. Also IGF-I can be bound but this is with ~10-fold lower affinity. IR-B has a high affinity for insulin, whereas affinity for IGFs is 100 times lower and therefore considered negligible. Though, the hybrid form of IR-B with IGF1R (HRB) can also

bind IGF-I with high affinity. Factors that contribute by switching from IR-B to IR-A in cancers are poorly understood. The amounts of expression of the 2 isoforms in tissues are different. IR-A is predominantly expressed in fetal and cancer cells. This might be due to expression of different oncogenes, inactivations of p53 and higher expression of HMGA proteins (which interact with transcription factors to promote activation of the IR gene). IR-B has metabolic effects mostly in the classic insulin sensitive tissues. There are also hybrid forms of IR-A and IR-B. HR-A is over-expressed in cancers. This HR-A has a broad ligand specificity; it can bind IGF-I and II and insulin. The HRB has not an important role and is possibly only involved in metabolic effects of insulin, just like the IR-B homodimer. (Pandini 2007, Rikhof 2008, Belifore 2008, Belifore review 2008, Weroha 2008, De Meyts 2004, Sacco 2009).

When insulin or IGF bind to a signal transducing receptor, autophosphorylation of the receptor leads to activation. Then, the intrinsic tyrosin kinase is activated and this allows phosphorylation of the adaptor protein, IRS. The expression of different IRS forms might be tissue specific and influences signaling. For example, IRS-1 is associated with proliferation, IRS-2 is associated with metastasis formation. The phosphorylation of the adaptor protein starts a cascade of signals by interaction with proteins with a SH2 domain. Two main pathways are involved, the Ras-raf-MEK-ERK and the PI3K (phosphatidylinositol-3-kinase)-Akt, which are both survival pathways. The ras-raf-MEK-ERK leads to proliferation and differentiation. After ligand binding to the receptor Ras is activated indirectly by IRS, via the growth factor receptor-bound protein 2 (Grb2), or directly via SHC. IRS and SHC compete for binding phosphorylated tyrosine residues. Active Ras activates raf. Consecutive, MEK and ERK1/2 are also activated. Also, MAP kinase (mitogen-activated protein kinase) is phosphorylated by Ras, and leads to activating of a several nuclear transcription factors. These activated nuclear transcription factors affects expression of genes involved in cell growth. (book pharmacology 2003). The other major pathway is the PI3K pathway. PI3K activates Akt (by phosphorylation). This occurs via binding of the PI3K p110 subunit on an IRS. Then, PIP2 is converted into PIP3 and phosphorylates Akt. Phosphorylated Akt has an important role in the regulation of glucose homeostasis and with protection against apoptosis. Next to that, Akt activates mTOR (mammalian target of rapamycin) and mTOR itself stimulates protein synthesis and cell growth. There is also evidence that PI3K foxOs (forkhead family of transcription factors) inhibits. Dubrovskaja et al show data of this in prostate cancer stem-like cells. Normally, foxOs have effects on processes like cell cycle arrest, apoptosis and DNA repair. FoxO3a has been identified as important factor in maintenance of unlimited life span in hematopoietic stem cells (23 van Dubrovskaja). When PI3K is activated, it phosphorylates and inactivates foxOs. Transcription does not occur in this way. Additionally, an increase in sphere-forming, higher clonogenic capacity and higher self-renewal was visible in foxO3a-knockdown prostate cancer cells (DU 145). Cells with the stem cell marker CD133+ had also high levels of phosphorylated foxO3a. These results also suggest an important role of foxOs, and therefore also PI3K, in high self-renewal character of cancers. The same study (Dubrovskaja et al) reported evidence for the reduction of PTEN, which normally inhibits PI3K. PI3K levels were therefore higher in stem-like cell populations which grow under sphere-forming conditions. Knocking-down of PTEN showed more tumorigenic potential. Additionally, P53 (inducer apoptosis) is down-regulated. Other

inhibitors of the PI3K pathway are AMPK and Rapamycin, which inactivate mTOR. (Pollak 2008 review, Dziadziuszko 2008, Sacco 2009, Rikhof 2008, book pharmacology 2003, Dubrovuska 2008, Gibson 2007).

The IRs have not been of interest for a long time, but they might also have a major role in the signaling of IGFs. IR up-regulation was demonstrated in breast, lung, colon, ovary and thyroid cancer. This was not due to amplification of the IR gene or to an rearrangement of the gene. Though, there was shown an up-regulation of the gene, due to mutations in several oncogenes (like wnt (oncogene), Neu and Ret). Also inactivating mutations were found, for example in p53. Normally, p53 suppresses the IR and IGF1R promoter activity. Consequently, loss of p53 resulted in a higher IR expression by increasing the IR promoter activity and by up-regulation of the IR protein expression. This was visible in thyroid cancer. IGF1R is also influenced by the loss of p53 but this is less visible than the effects on IR. Belifore et al (2007) describe data which show that in breast cancer 40 % of different cancer species had high IR and low IGF1R expression and 15% has low IR and high IGF1R expression. Equal data were found in cultured breast cancer cells. (Belifore, 2007). When IR was researched more closely, IR-A was determined as the most over-expressed form of IR in breast, colon, lung, thyroid and myosarcomas (Pollak 2008 review). Additionally, other data suggests that this spliced form of the IR is more responsive to IGF-II activation than the IR-B isoform. Knowledge about the basis and the importance of preferential expression of IR-A and its hybrid receptor in neoplasm is still an important subject of study. Results suggests that IGF-II binds to IR-A with the same affinity as to IGF1R. Additional, there are data that suggests that IGF-II even preferentially binds to IR-A in breast carcinoma. Next to that, in thyroid cancer is found that IGF-II binds stronger than insulin. (Pollak 2008 review and Weroha 2008). In the study of Sacco et al (2009) downstream signaling of IGF-I, II and insulin were investigated in IR-A + but IGF1R- fibroblasts (R-/IR-A cells). This study reported that IGF-II was more potent to activate p70S6 kinase (a kinase which plays a crucial role in growth and proliferation) than insulin. This activation of p70S6 is reported although IGF-II has a lower affinity (by less autophosphorylation of the IR-A receptor). IGF-II had approximately equally effects on activating early peaks of ERK1/2 and Akt as insulin. Notably, also IGF-I induced a similar signal as IGF-II, this despite the fact that it induces an even lower autophosphorylation of the IR-A. Because up-regulation of IGF1R and IR-A was visible in breast cancer, research to the content and function of the HR is done. Though, it is discovered that IR-A over-expression also leads to higher expression of HRs, and therefore facilitates more binding sites for IGFs. HR-A is the hybrid form that is up-regulated in cancers. This HR-A bind IGF-I and IGF-II with the same affinity as an IGF1R. HRs are over-expressed in several cancers like breast and thyroid cancer. Mainly, HR-A exists. HR-B can be formed but this does not occur often, and it is not up-regulated in cancers. In 75% of the specimens of human breast cancers the HRs exceeded the IGF1R expression. In some cells, IGF-I-HR exceeded the IGF-I-IGF1R, which suggests that most of the IGF-I effect occurs via HR. In breast cancer and thyroid cancer was found that the most present receptor gives the most mitogenic effect. This was concluded from the larger effect by blocking the most present receptors. Additional, in thyroid cancer was measured a higher expression of HR than predicted. This suggests that the cancer favors HRs more than homodimers.

Additional, HR-As increase insulin sensitivity because IR-A facilitated activation of the beta-subunit of the IGF1R after insulin binding. (Belifore, 2007 and Sacco 2009) In conclusion, the data from the earlier discussed studies suggest that IGF I and II induce significant downstream signaling when binding to HR-A, although they lead to a minimal IR-A autophosphorylation. This, taking together with the evidence that IR-A and HR-A are over-expressed in cancers, supports the concept that IR-A/HR-A should be investigated more (in response to IGFs) and that IR-A/HR-A blockade as anticancer therapies should be researched.

Therapeutic strategies to target IGF1R and IR

IGF1R has been investigated and methods of blocking IGF1R are already provide in many ways, whereas targeting IR has not been of interest for research for many years. Weroha et al (2008) describes a few manners for blocking the IGF1R. First, interruption of the receptor-ligand interaction as a result of adding IGF1R monoclonal antibodies, (mAbs) is often used (see figure 2). IGF1R mAbs are targeted against the extracellular part of the receptor and block the primary mitogenic signal and signal transduction of IGF-I and II. Besides, they down-regulate the expression of IGF1R and both his hybrid receptors HR-A and HRB. The major difference between the various mAbs is the half-life, which can be important in application of the treatment.

Examples of mAbs are AMG-479, MK0646, R1507 and CP 751,871. The last one is a new found biological substance, which has already been researched in combination with chemotherapy. In non small-cell lung cancer was found that this substance, combined with carboplatin and paclitaxel (chemotherapeutica) made the response rate increase from 41% (chemotherapy alone) to 54% (combination). In squamous cancers, chemotherapy in combination with CP 751,871 induced the response rate more significant, it changed from 41% to 72%. Several adverse effects were measured but these were acceptable. Early phase III studies will be performed to see if this could be used as a functional and relatively non-harmful cancer therapy (Weroha 2008).

Though, a disadvantage of mAbs is that they only bind IGF1R and not IR-A. Though it has effects on hybrids (HR-A) by binding on the IGF1R part and causes internalization. Apparently, affinity towards only one part of the heterodimere is enough to block the receptor. Pandini et al (2007) supports this by describing a neutralising antibody against IGF1R that had also (break-down) effects against his hybrids with IR (HRs). They described higher anti-tumoural responses when antibody h7C10 was added in a xenograft mouse model. However theoretical if insulin signaling via IR-A is strong enough (and there are enough HR-A and IR-A homodimers), it could overcome the IGF1R blockade. Noted must be that IR-B blocking is not a goal, because the IR-B plays a crucial role in the glucose metabolism of the cell. Classic insulin sensitive tissues that have high expression of IR-B, must continue their actions. The consequences of blocking the metabolic insulin effects are unknown and therefore more research has to be done before actions could be determined.

Nowadays it is thought that hybrid receptors provide additional binding sites for mitogenic signals of IGF-I and II and therefore it is the most important target. mAbs

for IGF1R could be used, for IRs down-regulation but IR-A is still available and can still cause a proliferative effect (Belifore 2007 and Weroha 2008). This is why IR-A mAbs are urgently needed in anti-cancer therapy.

An other method is the application of IGFBPs. IGFBPs acts as natural antagonists by forming high affinity IGFBP-IGF complexes and therefore limiting access to IGF1R. Secretion of IGFBPs is influenced by tumor suppressor gene p53, vitamin D, anti-estrogens, retinoids and TGFβ (Pollak 2008). The IGFBP3 form is the most presented IGFBP in the serum of the blood. Besides, in lung cancer it has been discovered that reduction of this cancer is associated with high concentrations of the IGFBP3 form (Dziadziuszko, 2008 and Rikhof 2008). IGFBPs might have anti-tumor opportunities because it (theoretical) lowers the concentration IGFs and therefore both IGF1R and IR have a lower signal transduction. Probably, insulin levels also have to be low, so insulin could not overcome the reduction in IGF induced-signaling by IGFBPs.

Pollak (2009) describes a contradiction in his review. Namely, over-expression of IGFBPs is not always associated with a decrease but sometimes even associated with an increase in IGF signaling. The mechanisms involved are not yet discovered but has to be investigated. One possibility is that IGFBPs have biological activities, independently of their IGF-binding properties. According to Rikhof (2008) there is evidence that IGFBPs themselves have antiapoptotic functions. The mechanism is still unknown. Considering the results from Dziadziuszko, Pollak, and Rikhof, IGFBP3 up-regulation might probably be used for cancer therapy. Yet, there is contradictory evidence about inactivation of IGFs by IGFBPs and therefore more research is required.

Weroha et al (2008) describe another manner to block the IGF signaling: ligand sequestration by the use of mAbs and IGFBPs (see figure 2). This method would have the potential benefits of the blockade and kinase inhibition but without negative effects of blocking IR-B (negative metabolic effects). Insulin could have mitogenic properties and therefore could overcome this blockade. This is why insulin has also to be in low concentrations in the blood.

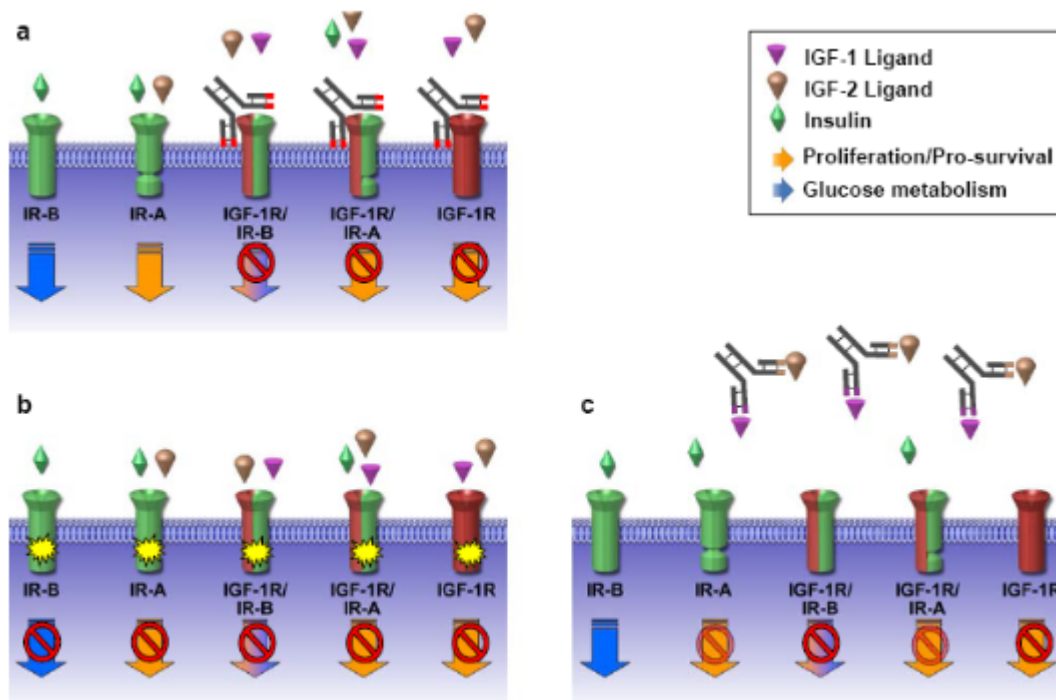


Figure 1 Model of IGF system inhibition strategies. a Monoclonal antibody strategy; b tyrosine kinase inhibitor strategy; c ligand sequestration strategy.

Figure 2 Model of IGF system inhibition strategies. a Monoclonal antibody strategy; b tyrosine kinase inhibitor strategy; c ligand sequestration strategy. (Weroha and Haluska, 2008)

Another strategy is the use of small molecule inhibitors, which are targeting the intracellular kinase domain instead of the extracellular binding. To date, small molecule inhibitor INSM 18 is the only non-mAb drug available for inhibition of the IGF-axis. This chemical inhibits specifically the IGF1R and has also activity against the HER2 receptor. Notice that some forms of breast cancer are associated with high HER levels; in 25% of all invasive breast cancers it is up-regulated (so called HER2-positive breast cancer). The study of Weroha (2008) shows data that INSM 18 has anti-tumoural activity in prostate cancer. Unfortunately, this is not performed in breast cancers yet. The mechanism is also still unknown and additional research has to be done to get more insights.

Specific tyrosine kinase inhibitors have good opportunities as anti-cancer therapy because they block all members of the IR and IGF families. Though, the study of Nielsen et al (2008) discusses disadvantages of the treatment. Namely, these inhibitors are less specific than antibodies and this can cause side effects and increase toxicity. So, a disadvantage of the application of specific tyrosine kinase inhibitors against IGF1R and IR is the unknown metabolic effects by blocking also the IR-B insulin signaling. (Weroha 2008 and Nielsen 2008).

Amplification or addition of soluble IGF2R could probably be used as anti-cancer therapy. IGF2R is identified as a tumor suppressor gene because it prevents binding of IGF-II to IGF1R and the receptor causes internalization if it is on the membrane. Also in many cancers, loss of IGF2R is found. Over-expression of the receptor is associated with less tumor growth and less tumor genesis. When IGF2R for the use of anticancer therapy is forced over-expressed, IGF-II is down-regulated. The main question is how important the IGF-II signaling is. For therapy, soluble IGF2R could

be an option. This method has to be used in combination with an IGF-I blocker and a partly insulin blocker (IR-A). (Rikhof 2008, Pollak 2008)

Medication directed to IRS-1 is also an option. There are 4 major forms of IRS; IRS-1, IRS-2, IRS-3 and IRS-4. IRS-1 and IRS-2 are the most important and are found in breast cancer. Gibson et al (2007) described its function as a metastasis inhibitor, whereas IRS-2 is discovered to be a metastasis inducer. This is supported by data that show IRS-1 is down-regulation in 40 % of NSCLC human tissue. IRS-2 is up-regulated in pancreatic and hepatocellular cancers. So, stimulators of IRS-1 formation or medication against IRS-2 must be determined and the treatment in cancers should be discovered. (Gibson 2007)

Discussion

First, novel therapeutic strategies for cancer treatment are urgently needed because of the high occurrence of this severe illness. More research has to be performed for insights in blocking methods of IGFs in anti-cancer therapies. Namely, the key role of (high expression of) IGF in the proliferation and progression of cancers is obvious because of the large scale of evidence for. It might be that IGF interruption only has effects on the resistance of cancer against chemotherapy. Knowing that resistance to chemotherapy is a major problem, reduction in resistance would be a step in the good direction of complete treatment of cancers.

Many researchers have reported that cancers express more IGF1R, IR-A, HR-A. Especially HR-A is an important target for anti-cancer therapy because it is found more over-expressed than IGF1R in cancers and it facilitates a shift from insulin to the IGF pathway by binding with high affinity to IGFs. Additionally, HR-A is the only receptor form which can bind all three hormones insulin, IGF-I.

Comparing the evidence and methods for blocking IGF1R, the treatment with antibodies, IGFBPs and small molecule inhibitors have opportunities to block also the IR- IGF binding. Considering the fact that there are no existing antibodies for IR-A yet, research to development of IR-A antibodies must be performed. Nowadays, until these are available, IGF1R- and IR-ligand interaction must be blocked in another way. The best treatment is by binding to IGF-ligand itself and additional also by binding the IGF1R and HRs (and also the IR-A when IR-A antibodies are discovered). Application of IGFBPs, which bind the ligand in combination with antibodies, which are added for interruption of the ligand-receptor binding, might accomplish this. Additionally, these two combined with small molecule inhibitors in low concentrations might also result in more sensitive therapeutics. The small molecule inhibitors has to be in low concentration, so the low levels that break through the IGF-receptor blockade are inhibited, but it is too low to interrupt the entire insulin signaling pathway in classic normal tissues. Additionally, low concentrations decrease the possible toxic effects of the non-specific character of tyrosine kinase inhibitors. Noted must be that if insulin itself has a strong proliferation effect, it could overcome the blockade. When IR-A antibodies are also discovered and added, the insulin itself has less effect because it can not bind IR-A but only bind IR-B, which is important in glucose metabolism.

There are no equal recommendations/ advices for the blockade of the IR-A/HR-A in all cancers as a result of the different expressions and characteristics of every cancer type. For example, small molecule inhibitor INSM-18 has preference for breast cancer, taking in consideration that high levels of HER2 are expressed in this cancer and INSM-18 has activity against IGF1R and against HER2. In combination with existing chemotherapy it could elevate responses to chemotherapy. For lung cancer there is evidence that CP751,871 (antibody against IGF1R) in combination with chemotherapy causes higher response rates but it is still not optimal (from 41% chemotherapy alone and 54% in the combination). It might be important that the IR-A is also blocked. This can be done by addition of small molecule inhibitors in low concentrations.

Concluding, more research has to be done to how this HR-A and IR-A can be blocked totally. Overall, IR-A blocking antibodies should be developed especially when treatment with tyrosine kinase inhibitors cause high toxicological effects. IR-A

antibodies could then used in treatment together with IGF1R antibodies and IGFBPs against IGF1R, IR-A and HR-A in IGF signaling.

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