

Review of: Inhibition of *in vivo* breast cancer growth by antisense oligodeoxynucleotides to type I insulin-like growth factor receptor mRNA involves inactivation of ErbBs, PI-3K/Akt and p42/p44 MAPK signalling pathways but not modulation of progesterone receptor activity

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Abstract of the original article

The present study addresses the effect of targeting type I insulin-like growth factor receptor (IGF-IR) with antisense strategies in *in vivo* growth of breast cancer cells. Our research was carried out on C4HD tumors from an experimental model of hormonal carcinogenesis in which the synthetic progestin medroxyprogesterone acetate (MPA) induced mammary adenocarcinomas in Balb/c mice. We employed two different experimental strategies. With the first one we demonstrated that direct intratumor injection of phosphorothioate antisense oligodeoxynucleotides (AS[S]ODNs) to IGF-IR mRNA resulted in a significant inhibition of C4HD tumor growth. In the second experimental strategy, we assessed the effect of intravenous (i.v.) injection of AS[S]ODN on C4HD tumor growth. This systemic treatment also resulted in significant reduction in tumor growth. The antitumor effect of IGF-IR AS[S]ODNs in both experimental protocols was due to a specific antisense mechanism, since growth inhibition was dose-dependent and no abrogation of tumor proliferation was observed in mice treated with phosphorothioate sense ODNs (S[S]ODNs). In addition, IGF-IR expression was inhibited in tumors from mice receiving AS[S]ODNs, as compared to tumors from control groups. We then investigated signal transduction pathways modulated *in vivo* by AS[S]ODNs treatment. Tumors from AS[S]ODN-treated mice of both intratumoral and i.v. protocols showed a significant decrease in the degree of insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation. Activation of two of the main IGF-IR signaling pathways, phosphatidylinositol 3-kinase (PI-3K)/Akt and p42/p44 mitogen-activated protein kinases (MAPK) was abolished in tumors growing in AS[S]ODN-treated animals. Moreover, ErbB-2 tyrosine phosphorylation was blocked by *in vivo* administration of AS[S]ODNs. On the other hand, we found no regulation of either progesterone receptor expression or activity by *in vivo* AS[S]ODNs administration. Our results for the first time demonstrated that breast cancer growth can be inhibited by direct *in vivo* administration of IGF-IR AS[S]ODNs.

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Review

Insulin-like growth factors (IGFs) are critical regulators of normal cell growth. This has been shown in animal models and in human diseases, where disruption of

IGF signalling results in dwarfism. However, as often happens in life, too much of a good thing can be bad. Thus, the potent proliferative and survival signals elicited by the IGFs are perfect for cancer growth and progression [1]. Indeed, it has been shown that IGF-I receptor (IGF-IR) is absolutely required for maintenance of the transformed phenotype [2]. The evidence that IGFs are key to tumour growth and progression has led to the IGF-IR becoming a major therapeutic target in cancer [3]. Several strategies to inhibit IGF-IR are in preclinical and clinical development, and the next 5 years will be pivotal in determining the applicability of anti-IGF strategies in cancer management and treatment.

Elizalde's group has been studying a mammary tumour (C4HD) that arose in synthetic progestin (methoxyprogesterone acetate (MPA)) treated Balb/c mice. The group has shown that this tumour is stimulated by MPA *in vitro* and *in vivo* [4]. This proliferation is driven by a complex interaction between progestins and growth factors. For example, MPA can upregulate levels of heregulin (HRG) and its receptor ErbB2 [5], and results in activation of an ErbB2/ErbB3 complex. Blockade of HRG by antisense oligodeoxynucleotides (ASODN) abrogates the effect of MPA on C4HD cells, thus directly implicating HRG in the MPA-mediated proliferation. Interestingly, C4HD cells also express IGF-IR and secrete IGF-I and IGF-II, and ASODNs to IGF-II can also block MPA-induced proliferation [6].

A recent paper by Salatino *et al.* [7] describes the first application of antisense IGF-IR oligonucleotides to block mammary cancer growth *in vivo*. They showed that ASODN to IGF-IR efficiently block MPA-induced proliferation of C4HD cells *in vitro*, and that direct intratumor injection of ASODNs can also block tumour growth *in vivo*. They went on to show that intravenous (i.v.) administration of ASODNs can block the proliferation of C4HD tumours. This blockade of tumour growth was associated with a reduction in mitosis and the appearance of necrosis. Analysis of signalling within the tumours indicated that ASODN to IGF-IR caused a decrease in the levels and phosphorylation of IGF-IR, and that this was associated with decreased phosphorylation of insulin receptor substrate-1 (IRS-1), Akt and ERK1/2. They therefore concluded that the IGF-IR signalling pathway had been effectively blocked.

While the effect of i.v. ASODNs to IGF-IR further strengthens the rationale for IGF-IR as an excellent target for inhibition of breast cancer growth, one of the most intriguing aspects of this research is the apparent cross-talk between ErbB1/2 and IGF-IR. Several laboratories have noted bi-directional cross-talk between these receptors, with the term 'signal transactivation' appropriately describing this form of

lateral signalling [8]. This was first described in IGF-IR null fibroblasts, which fail to respond to EGF and place IGF-IR as an upstream regulator of ErbB1 [9]. Similarly, Balana *et al.* [4] showed that in C4HD cells, ASODNs to IGF-IR blocked HRG action, and that there was a physical association between ErbB2 and IGF-IR. This same association was also found in MCF-7 human breast cancer cells and could be induced by either HRG or IGF-I. The authors summarized that there was a 'hierarchical interaction' between IGF-IR and HER-2 [4]. Salatino have now shown that this interaction can also be found in the C4HD tumours grown *in vivo*, and that ASODNs to IGF-IR can disrupt ErbB2/3 association and phosphorylation [7].

This is the first *in vivo* evidence that an anti-IGF-IR strategy can inhibit ErbB2/3 activity and clearly has important implications in breast cancer treatment. The interaction and bi-directional cross-talk between ErbB receptors and IGF-IR has been documented in a number of different cell systems (and now *in vivo*) and needs to be taken into account when considering either of these receptors as targets for blocking breast cancer progression. In this regard, it has already been shown that overexpression of IGF-IR can cause resistance to ErbB1 [10] or ErbB2 [11] inhibition. Given that IGF-IR seems to be an upstream regulator of ErbB action, this would make co-targeting of IGF-IR and ErbB1/2 an absolutely essential strategy for efficient ErbB1/2 blockade. In this regard, the recent reported bi-specific ErbB1/IGF-IR human monoclonal blocking antibody (ImClone Systems Incorporated, New York, NY) may be especially attractive [12].

Another important clinical aspect of the MPA-induced C4HD tumours is their progression to hormone independence. Similar to other models using human breast cancer cells, progression to hormone independence in C4HD cells seems to involve a complex interaction between PR, IGF-IR, and EGFR/HER-2, with independence being associated with high constitutive levels of both HRG and IGF-II, and ASODNs to either of these ligands blocking the growth of C4HD cells [5,6].

Several critical questions remain. First, what is the relevance of progestin-induced mammary cancer to human disease? Progesterone and its receptor (PR) remain a relatively understudied area in human breast cancer, often playing second fiddle to the oestrogen receptor (ER). However, recent hormone replacement therapy studies have clearly shown a role for progesterone in breast cancer, and recent clinical data regarding the utility of PR in predicting anti-oestrogen resistance is starting to cause re-appreciation of the role of progesterone and PR in breast cancer [13]. Second, is the PR cross-talk

related or similar to ER cross-talk with growth factors? Can the last decade of research on cross-talk between ER and growth factors be used to rapidly elucidate cross-talk with PR? Third, are ASODNs viable therapeutic strategies for cancer treatment? Finally, what are the exact molecular mechanisms for cross-talk between ErbB1/2 and IGF-IR? Sophisticated biochemical analysis needs to be performed to understand how these receptors associate, and who is activating whom. Newly available IGF-IR and ErbB1/2 inhibitors will surely aid in this effort.

In summary, Salatino *et al.*'s report provides further exciting evidence that IGF-IR is critical for mammary cancer growth, and that IGF-IR may be an upstream regulator of ErbB1/2 action. The accumulation of *in vitro* and *in vivo* evidence implicating IGF-IR as a therapeutic target will be tested in the next 5 years with the clinical advancement of various strategies to target this receptor. It is hoped that this endeavour will be fruitful and will provide a new weapon in the fight against breast cancer.

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