Neutralizing Anti-Insulin-like Growth Factor Receptor 1 Antibodies Inhibit Receptor Function and Induce Receptor Degradation in Tumor Cells

Judith Hailey, Eugene Maxwell, Kathy Koukouras, W. Robert Bishop, Jonathan A. Pachter, and Yan Wang[1]

Department of Tumor Biology, Schering-Plough Research Institute, Kenilworth, New Jersey 07033

Abstract

Insulin-like growth factor receptor 1 (IGFR1) plays a crucial role in oncogenic transformation [C. Sell *et al.,* Mol. Cell. Biol., *14:* 3604–3612, 1994]. Compared with the normal human mammary epithelial cell line MCF12A, MCF7 human mammary carcinoma cells overexpress IGFR1 on the cell surface. To measure the effects of IGFR1 inhibition on tumor cells, we tested two mouse neutralizing antibodies against human IGFR1 in cell-based assays. Both MAB391 and anti-IR3 antibodies inhibit IGFR1 autophosphorylation upon IGF-I ligand stimulation with IC_{50}s of 0.58 and 0.80 nM, respectively. When cells were treated with neutralizing anti-IGFR1 antibodies for ≥4 h, the total receptor level was dramatically decreased. IGF-I-stimulated activation of AKT was also inhibited by anti-IGFR1 antibodies. Furthermore, MAB391 and anti-IR3 inhibited the growth of MCF7 cells in soft agar. In addition to MCF7 cells, MAB391 also inhibited IGFR1 autophosphorylation and induced IGFR1 down-modulation in HT29 colorectal and Du145 prostate cancer cells. Therefore, neutralizing antibodies against IGFR1 represent a valid approach to inhibit growth of tumor cells.

Introduction

IGFs[2] stimulate the proliferation of cultured human breast cancer cells (1). This stimulation is mediated through the receptor IGFR1, which is a member of the receptor tyrosine kinase family (2, 3). When activated by its ligands (IGF-I or IGF-II), IGFR1 phosphorylates tyrosine residues on two major substrates, IRS-1 and Shc, which subsequently signal through the Ras/Raf and phosphatidylinositol 3′-kinase/AKT pathways. IGFR1 plays a crucial role in transformation. Cells derived from IGFR1 knockout mice are resistant to transformation by various viral and cellular oncogenes, including SV40 large T antigen and activated ras, whereas fibroblast cells from wild-type mice can be readily transformed by these oncogenes (4).

There is increasing epidemiological evidence to link elevated plasma IGF-I level with prostate, breast, and colon cancer risk (5–7). Breast cancer tissues from patients exhibit higher IGFR1 expression than adjacent normal tissue, suggesting a link between IGFR1 and breast epithelial cell transformation (8, 9). It has been reported that the transformation capacity of tumor cells is attenuated when IGFR1 is inhibited using an antisense strategy, neutralizing antibody (anti-IR3) or dominant negative truncation of the receptor (10–18).

To further investigate the antitumor activity and mechanism of anti-IGFR1 antibodies, we used two neutralizing antibodies against IGFR1 and tested them in cell-based assays. Our data show that neutralizing antibodies against IGFR1 can efficiently inhibit receptor autophosphorylation and downstream activation of AKT by a mechanism that includes induction of receptor degradation. This antibody-induced disruption of IGFR1 signaling results in inhibition of anchorage-independent growth of tumor cells. Therefore, generation of humanized or human neutralizing antibodies against IGFR1 may represent a valid approach to inhibit tumor growth.

Materials and Methods

Cells and Reagents. MCF7 human breast carcinoma cells, HT29 colorectal cancer cells, and Du145 prostate cancer cells are from American Type Culture Collection. MAB391 is purchased from R&D Systems, and anti-IR3 is from Oncogene Science. Antibodies against total AKT and phospho-AKT (serine 473) are obtained from Cell Signaling, and anti-Actin antibody is from Sigma. Anti-IGFR1 antibody C-20 is purchased from Santa Cruz Biotechnology, and antiphosphotyrosine antibody 4G10 is from Upstate Biotechnology. ECL developing solutions are from Amersham.

Receptor Autophosphorylation Assay. Antibodies were added to cells for various lengths of times. Cells were then stimulated with 10 ng/ml IGF-I for 5 min at 37°C. Cells were washed twice with cold PBS containing 0.1 mM sodium vanadate and lysed in lysis buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, protease inhibitors, and 2 mM sodium vanadate). Lysates were incubated on ice for 30 min and then centrifuged at 13,000 rpm for 10 min at 4°C. Protein concentrations of the lysates were measured by Coomassie Plus Protein Assay (Pierce). Lysates were then subjected to immunoprecipitation and Western blot analysis.

Soft Agar Assay. Three ml of 0.6% agarose in complete MEM were added to each well of 6-well tissue culture plates and allowed to solidify (bottom layer). One hundred μl of MAB391 antibody at various concentrations was added to

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[1] To whom requests for reprints should be addressed, at Department of Tumor Biology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033.

[2] The abbreviations used are: IGF, insulin-like growth factor; IGFR1, IGF receptor 1; ECL, enhanced chemiluminescence.

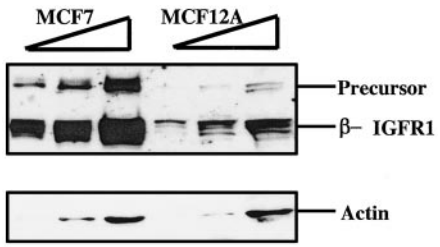

Fig. 1. Overexpression of IGFR1 in MCF7 human breast cancer cells. Equal amounts of cell lysates from MCF7 and normal control MCF12A cells (three levels for each line) were analyzed by Western blot. The filter was probed with an anti-IGFR1 antibody and detected by ECL. The same blot was then stripped and probed with an antiactin antibody as a control.

culture tubes. Aliquots of MCF7 cells (15,000 cells) were added to the culture tubes containing antibody and incubated at room temperature for 10–15 min. Three ml of a 0.35% agarose/complete MEM layer (top layer) were added to the antibody/cell mixture and then plated onto the solidified bottom layer. The top layer was allowed to solidify. The plates were then incubated for 3 weeks. 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide was added to the wells and incubated for 1–2 h. The plates were scanned with an adapted Hewlett Packard Scanjet, and the colonies counted and analyzed using a customized colony counter application program.

Immunoprecipitation and Western Blot Analysis. IGFR1 was immunoprecipitated using 1 μg of antibody (C-20; Santa Cruz Biotechnology) and 15 μl of 50% protein G agarose. The precipitates were washed four times with lysis buffer. The washed precipitated proteins were separated on 10% SDS-PAGE, transferred to a nitrocellulose filter, probed with either anti-IGFR1 or antiphosphotyrosine (4G10) antibodies, and visualized by ECL (Amersham).

For direct Western blot analysis, equal amounts of cell lysates were separated on 10% SDS-PAGE, transferred to nitrocellulose filters, probed with desired antibody, and visualized by ECL (Amersham).

Results

Overexpression of IGFR1 in Breast Cancer Cells. Using Western blot analysis, we investigated the expression levels of IGFR1 in various breast cancer cell lines. Among the lines that we examined, 40% exhibited elevated IGFR1 expression (data not shown). The estrogen-sensitive breast cancer cell line MCF7 had the highest expression level (Fig. 1). Compared with the immortalized normal breast epithelial cell line MCF12A, MCF7 showed 10-fold overexpression. The receptor number on MCF7 cell surface has been estimated at 43,000 by Scatchard analysis of IGF-I binding assay (data not shown). Therefore, we used the MCF7 cell line for additional analysis.

Inhibition of Receptor Autophosphorylation. Because the MAB391 and anti-IR3 antibodies have been reported to be neutralizing, we determined their effect on IGF-I-stimulated receptor autophosphorylation. Cells were treated with the antibodies for 1.0 or 1.5 h before they were stimulated with 10 or 20 ng/ml IGF-I ligand for 5 min. Phosphorylation of IGFR1 was inhibited in a dose-dependent manner by treatment with MAB391 with an IC_{50} of 0.58 nM in MCF7 cells (Fig. 2, *A* and *B*). The anti-IR3 antibody had a similar effect (data not shown). An isotype-matched mouse IgG1 had no effect on receptor phosphorylation (negative control). Another anti-IGFR1 mouse monoclonal antibody 3B7 (Santa Cruz Biotechnology), which is known not to be neutralizing, also did not inhibit IGFR1 autophosphorylation (data not shown). The inhibition observed with MAB391 can be reversed by addition of excess IGF-I ligand (Fig. 2*C*), suggesting that the antibody inhibits phosphorylation in a manner that is competitive with IGF-I ligand. We have also tested the effect of MAB391 on receptor autophosphorylation in HT29 colorectal cancer cells and Du145 prostate cancer cells. Data in Fig. 2, *D* and *E*, show that phosphorylation of the receptor in both lines was inhibited in a dose-dependent manner by the antibody.

Inhibition of AKT Phosphorylation and Induction of IGFR1 Down-Modulation. IGFR1 is known to signal through phosphatidylinositol 3′-kinase and phosphoinositide-dependent kinase 1-dependent phosphorylation of AKT on threonine 308 and serine 473 (19, 20). To investigate the effect of neutralizing antibodies on the downstream targets of IGFR1, we examined the phosphorylation of AKT. MCF7 cells were incubated with MAB391 for 20 h before they were stimulated with 10 ng/ml IGF-I for 5 min. Cell lysates were prepared and subjected to Western blot analysis. Long-term incubation with the neutralizing antibody inhibited phosphorylation of AKT (*top panel*, Fig. 3*A*) without affecting the total expression level of the AKT protein (*bottom panel*, Fig. 3*A*). Unexpectedly, this long-term treatment caused down-modulation of the IGFR1 receptor (Fig. 3*B*). The anti-IR3 antibody had the same effect on receptor down-regulation (data not shown).

Degradation of the IGFR1 Is Dose Dependent and Partially Lysosome Dependent. To further study the down-regulation of the IGFR1 receptor by neutralizing antibodies, we conducted a time course experiment. MCF7 cells were treated with MAB391 (20 nM), IgG1 control (20 nM), or IGF-I ligand (15 ng/ml) for various times, and IGFR1 levels were measured by Western blot analysis (Fig. 4*A*). By 4 h, the anti-IGFR1 neutralizing antibody induced significant down-regulation of the receptor. Over the same time period, the IGF-I ligand did not cause any measurable decrease in total receptor protein. The down-modulation of the receptor induced by the antibody is dose dependent (Fig. 4*B*). Similar dose-dependent receptor down-modulation was also observed in HT29 colorectal cancer cells and Du145 prostate cancer cells (Fig. 4, *C* and *D*). To examine whether IGFR1 expression is down-regulated by extended antibody treatment at the transcription level, we made RNAs from cells that were treated with MAB391 for 20 h and analyzed the RNA samples by Taqman. The quantitative PCR data indicated that the IGFR transcription level was not changed after 20 h of treatment of MAB391 at concentrations up to 100 nM (data not shown).

Because IGFR1 is internalized and degraded via a lysosome-dependent pathway (21), we tested the effects of inhibitors of lysosomal function or intracellular trafficking on anti-IGFR1 antibody-induced IGFR1 down-regulation. Among the reagents tested, chloroquine and dansylcadaverine (Fig. 4*E*) partially inhibited IGFR1 degradation. Brefeldin

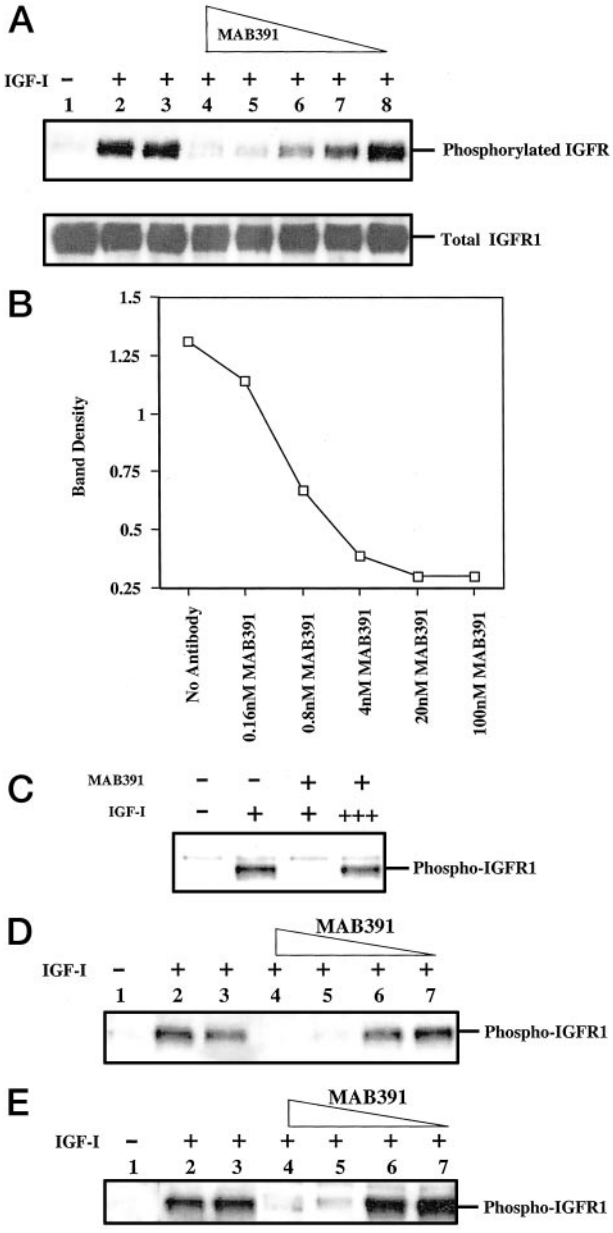

Fig. 2. Inhibition of receptor autophosphorylation upon IGF-I stimulation. *A*, MCF7 cells were treated with IGF-I (10 ng/ml) and various concentrations of MAB391 antibody (*Lane 4:* 100 nM; *Lane 5:* 20 nM; *Lane 6:* 4 nM; *Lane 7:* 0.8 nM; and *Lane 8:* 0.16 nM) for 1.5 h. A total of 400 μg of each cell lysate was subjected to immunoprecipitation with an IGFR1-specific antibody. The precipitated cell lysates were separated on 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with antiphosphotyrosine antibody 4G10. *Lane 1* is untreated cells. *Lane 2* is IGF-I (10 ng/ml)-treated cells. *Lane 3* is cells treated with IGF-I (10 ng/ml) and IgG1 (100 nM) control antibody. *B*, graphic representation of the data shown in *top panel A* (*Lane 2* and *Lanes 4–8*). The bands on the Western blot were scanned and quantified by Quantity One (PDI). *C*, MCF7 cells were treated with 10 nM MAB391 in the presence of 10 ng/ml (*Lane 3*) or 700 ng/ml *(Lane 4*) IGF-I ligand for 1.5 h. A total of 400 μg of each cell lysate was subjected to immunoprecipitation with IGFR1-specific antibody. The precipitated cell lysates were separated on 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with antiphosphotyrosine antibody 4G10. *Lane 1* is untreated cells. *Lane 2* is IGF-I (10 ng/ml)-treated cells. *D*, HT29 cells were treated with IGF-I (20 ng/ml) and various concentrations of MAB391 antibody (*Lane 4:* 20 nM; *Lane 5:* 4 nM; *Lane 6:* 0.8 nM; and *Lane 7:* 0.16 nM) for 1.5 h. A total of 400 μg of each cell lysate was subjected to immunoprecipitation with an IGFR1-specific antibody. The precipitated cell lysates were separated on 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with antiphosphotyrosine antibody 4G10. *Lane 1* is untreated cells. *Lane 2* is IGF-I (20 ng/ml)-treated cells. *Lane 3* is cells treated with IGF-I (20 ng/ml) and IgG1 (20 nM) control antibody. *E*, Du145 cells were treated with IGF-I (20 ng/ml) and various concentrations of MAB391 antibody (*Lane 4:* 20 nM, *Lane 5:* 4 nM; *Lane 6:* 0.8 nM, and *Lane 7:* 0.16 nM) for 1.5 h. A total of 400 μg of each cell lysate was subjected to immunoprecipitation with an IGFR1-specific antibody. The precipitated cell lysates were separated on 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with antiphosphotyrosine antibody 4G10. *Lane 1* is untreated cells. *Lane 2* is IGF-I (20 ng/ml)-treated cells. *Lane 3* is cells treated with IGF-I (20 ng/ml) and IgG1 (20 nM) control antibody.

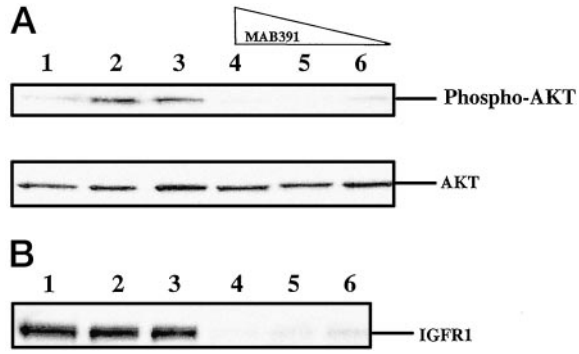

Fig. 3. Inhibition of AKT activation and induction of IGFR1 degradation. *A*, MCF7 cells were treated with IGF-I (10 ng/ml) and various concentrations of MAB391 antibody (*Lane 4*: 100 nM; *Lane 5:* 20 nM; and *Lane 6:* 4 nM) overnight. A total of 50 μg of each cell lysate was separated by a 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was first probed with an antiphospho-AKT antibody (*top panel*). Then the blot was stripped and reprobed with an anti-AKT antibody (*bottom panel*). *Lane 1* is untreated cells. *Lane 2* is IGF-I (10 ng/ml)-treated cells. *Lane 3* is cells treated with IGF-I (10 ng/ml) and IgG1 (100 nM) control antibody. *B*, the blot was stripped once again and reprobed with anti-IGFR1 antibody.

A or the proteasome inhibitors ALLN and MG132 did not affect receptor degradation induced by anti-IGFR1 neutralizing antibodies (data not shown). The down-modulation of the receptor was also unaffected in the presence of protein synthesis inhibitor cyclohexamide (Fig. 4F).

Inhibition of Anchorage-independent Growth. Ability to grow independent of attachment is one hallmark of cancer cells. IGFR1 null cells are resistant to transformation. Because anti-IGFR1 neutralizing antibodies are able to attenuate the signal cascade from IGFR1 by inhibiting receptor autophosphorylation (Fig. 2) and inducing receptor degradation (Fig. 3 and 4), we investigated the effect of the neutralizing antibodies on anchorage-independent growth of MCF7 cells. Consistent with previously published data (15), both MAB391 (Fig. 5) and anti-IR3 (data not shown) can inhibit the growth of MCF7 cells in soft agar in the presence of serum. The isotype matched IgG1 control does not have any inhibitory effect.

Discussion

There are several lines of evidence indicating that the IGF-I/IGFR1 system plays a role in breast cancer: (*a*) IGF-I is a potent transforming and mitogenic agent. It is locally produced by breast stromal cells (1). (*b*) IGFR1 is overexpressed on breast cancer cells (8, 9). (*c*) Antiestrogen therapy for

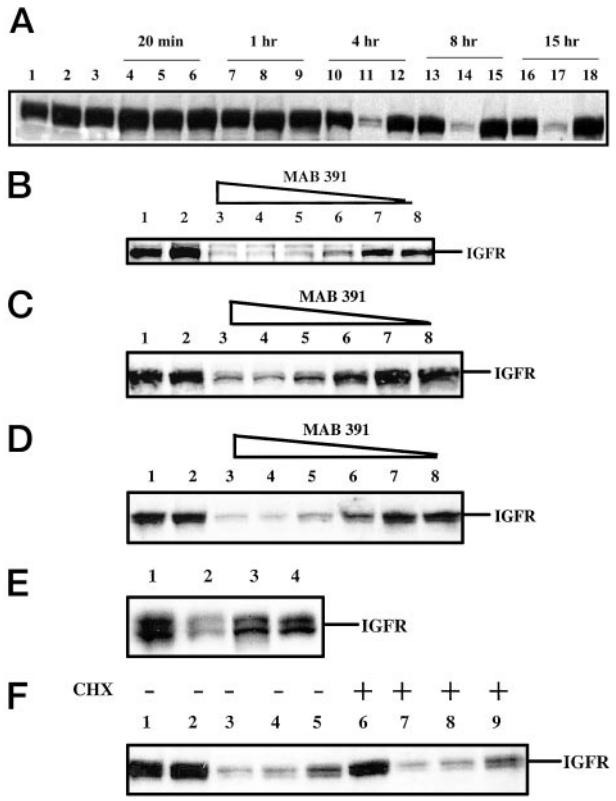

Fig. 4. Degradation of the IGFR1 is dose dependent and partially lysosome dependent. *A*, MCF 7 cells were treated for various times with 20 nM IgG1 (*Lanes 4*, *7*, *10*, *13*, and *16*), 20 nM MAB391 (*Lanes 5*, *8*, *11*, *14*, and *17*), or 15 ng/ml IGF-I (*Lanes 6*, *9*, *12*, *15*, and *18*). A total of 50 μg of each cell lysate was separated by 10% SDS-PAGE and transferred to a nitrocellulose filter. The filter was probed with anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lane 2* is cells that were treated with IGF-I (15 ng/ml) for 5 min. *Lane 3* is cells that were treated with 20 nM nonneutralizing anti-IGFR1 antibody 3B7 for 4 h. *B*, MCF7 cells were treated with various concentrations of MAB391 antibody (*Lane 3:* 100 nM; *Lane 4:* 20 nM; *Lane 5:* 4 nM; *Lane 6:* 0.8 nM; *Lane 7:* 0.16 nM; and *Lane 8:* 0.032 nM) overnight. A total of 50 μg of each cell lysate was separated by a 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lane 2* is cells that were treated with control IgG1 (100 nM) overnight. *C*, HT29 cells were treated with various concentrations of MAB391 antibody (*Lane 3:* 20 nM; *Lane 4:* 4 nM; *Lane 5:* 0.8 nM; *Lane 6:* 0.16 nM; *Lane 7:* 0.032 nM; and *Lane 8:* 0.006 nM) overnight. A total of 50 μg of each cell lysate was separated by 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lane 2* is cells that were treated with control IgG1 (20 nM) overnight. *D*, Du145 cells were treated with various concentrations of MAB391 antibody (*Lane 3:* 20 nM; *Lane 4:* 4 nM; *Lane 5:* 0.8 nM; *Lane 6:* 0.16 nM; *Lane 7:* 0.032 nM; and *Lane 8:* 0.006 nM,) overnight. A total of 50 μg of each cell lysate was separated by a 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lane 2* is cells that were treated with control IgG1 (20 nM) overnight. *E*, MCF7 cells were treated with chloroquine (400 μM, *Lane 3*) or dansylcadaverine (400 μM, *Lane 4*) for 30 min before 10 nM MAB 391 was added. Cells were incubated for additional 3 h. A total of 50 μg of each cell lysate was separated on a 10% SDS-PAGE, transferred to a nitrocellulose filter, and probed with an anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lane 2* is cells incubated with 10 nM MAB391 for 3 h. *F*, MCF7 cells were treated with various concentrations of MAB391 antibody (*Lanes 3* and *7:* 20 nM; *Lanes 4* and *8:* 4 nM; and *Lanes 5* and *9:* 0.8 nM) overnight in the absence (*Lanes 2–5*) and presence (*Lanes 6–9*) of 1 μg/ml cyclohexamide. A total of 50 μg of each cell lysate was separated by 10% SDS-PAGE and transferred to a nitrocellulose blot. The filter was probed with anti-IGFR1 antibody. *Lane 1* is untreated cells. *Lanes 2* and *6* are cells that were treated with control IgG1 (20 nM) overnight.

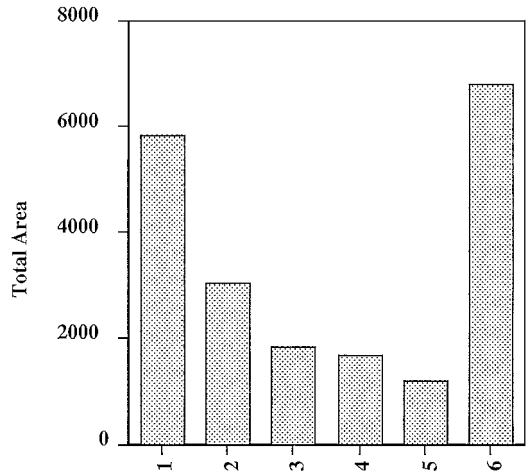

Fig. 5. Inhibition of anchorage-independent growth of MCF7 cells. MCF7 cells together with various amounts of MAB391 antibody (*Lane 2:* 0.8 nM; *Lane 3:* 4 nM; *Lane 4:* 20 nM; and *Lane 5:* 100 nM) were added into the top layer of agar. Cells were incubated in soft agar at 37°C for 3 weeks before staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The total areas of the colonies were measured by a scanner with an adapted Hewlett Packard Scanjet and analyzed using a customized colony counter application program. The average of duplicate values is shown here. *Lane 1* is untreated cells, and *Lane 6* is a mouse IgG1 negative control (100 nM).

breast cancer may achieve some of its antitumor effect by modulating the growth hormone (GH):IGF-I axis. For example, tamoxifen administration causes a reduction in serum IGF-I concentration by inhibiting both growth hormone secretion and local production of IGF-I (19). Antiestrogens also down-regulate IGFR1 receptor expression. Therefore, the IGF-I/IGFR1 ligand-receptor paracrine loop has been the target of a variety of strategies to inhibit cancer cell growth.

Various ways of inhibiting the IGF-I/IGFR1 axis have been documented in the literature. Antisense, dominant negative truncation or anti-IR3 antibody all inhibited the growth of cancer cells in soft agar and tumor formation in mice (10–18). We are interested in investigating the effects of anti-IGFR1 neutralizing antibodies on IGFR1 receptor signaling. We have shown that short-term treatment with anti-IGFR1 neutralizing antibodies inhibited receptor autophosphorylation (Fig. 2) and that the long-term treatment induced receptor degradation (Fig. 3). The antibodies also inhibited the phosphorylation of AKT, a downstream antiapoptotic signaling component of the IGFR1 pathway (Fig. 3). Phosphorylation of AKT on threonine 308 and serine 473 has been shown to correlate with AKT activation (20, 21). The effectiveness of anti-IGFR1 antibodies to inhibit MCF7 cell growth in soft agar in the presence of serum (Fig. 5), with no extra IGF addition, illustrates the critical dependence of breast cancer cells on IGF stimulation. In addition to the MCF7 breast cancer cells, we have also observed similar effects of anti-IGFR1 neutralizing antibodies on HT29 colorectal cancer cells and Du145 prostate cancer cells.

Receptor internalization is a process by which cell surface receptors are rapidly partitioned into intracellular vesicles. IGF-I binding initiates the migration of IGFR1 to clathrin-coated pits and the subsequent formation of early endosomes containing

internalized but still active receptors. The ligand-receptor complex ultimately becomes dissociated and inactivated in the acidic environment of late endosomes where ligands and receptors are sorted for degradation in lysosomes or recycling to the cell surface (22, 23). The data presented here indicate that long-term treatment with anti-IGFR1 neutralizing antibodies causes receptor degradation. This down-modulation was inhibited only partially by the lysosome inhibitors chloroquine or dansylcadaverine (Fig. 4). There are reports that some ligand-receptor complexes, including the highly homologous IGFR family member insulin receptor, internalize and are degraded via more than one pathway, including both a chloroquine-sensitive lysosomal pathway and a chloroquine-insensitive pathway (24). Our data suggest that IGFR1 is also internalized and degraded by a combination of both lysosome-dependent and lysosome-independent pathways. In contrast, proteasome inhibitors do not affect the pathway.

Both anti-HER2 and anti-EGFR antibodies have been shown to induce receptor internalization and down-regulation (25–27). Herceptin has been approved for treatment of breast cancer patients whose tumors overexpress Her2 receptor. The data presented here indicate that similar humanized or human neutralizing antibodies against IGFR1 would provide a valid approach to inhibit cancer cell growth. In addition, the expression of IGFR1 on many tumor cell types suggests that anti-IGFR1 therapy has the potential to treat a different and broader population of cancer patients than Herceptin.

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References

1. D'Ercole, A. J. Insulin-like growth factors and their receptors in growth. Growth Disord., *25:* 573–590, 1996.

2. Butler, A. A., Yakar, S., Gewolb, I. H., Karas, M., Okubo, Y., and LeRoith, D. Insulin-like growth factor-I receptor signal transduction: at the interface between physiology and cell biology. Comp. Biochem. Physiol., *121:* 19–26, 1998.

3. Sepp-Lorenzino, L., Structure and function of the insulin-like growth factor I receptor. Breast Cancer Res. Treat., *47:* 235–253, 1998.

4. Sell, C., Dumenil, G., Deveaud, C., Miura, M., Coppola, D., DeAngelis, T., Rubin, R., Efstratiadis, A., and Baserga, R. Effect of a null mutation of the type 1 IGF receptor gene on growth and transformation of mouse embryo fibroblasts. Mol. Cell. Biol., *14:* 3604–3612, 1994.

5. Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science (Wash. DC), *279:* 563–566, 1998.

6. Ma., J., Pollak, M., Giovannucci, E., Chan, J. H., Tao, Y., Hennekens, H., and Stampfer, M. J. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J. Natl. Cancer Inst. (Bethesda), *91:* 620–625, 1999.

7. Vadgama, J. V., Wu, Y., Datta, G., Khan, H., and Chillar, R. Plasma insulin-like growth factor-I and serum IGF-binding protein 3 can be associated with the progression of breast cancer, and predict the risk of recurrence and the probability of survival in African-American and Hispanic women. Oncology, *57:* 330–340, 1999.

8. Resnik, J. L., Reichart, D. B., Huey, K., Webster, N. J. G., and Seely, B. L. Elevated Insulin-like growth factor I receptor autophosphorylation and kinase activity in human breast cancer. Cancer Res., *58:* 1159–1164, 1998.

9. Pekonen, F., Partanen, S., Makinen, T., and Rutanen, E. Receptor for epidermal growth factor and insulin-like growth factor I and their relation to steroid receptors in human breast cancer. Cancer Res., *48:* 1343–1347, 1988.

10. Burfeind, P., Chernicky, C. L., Rininsland, F., Ilan, J., and Ilan, J. Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells *in vivo*. Proc. Natl. Acad. Sci. USA, *93:* 7263–7268, 1996.

11. Neuenschwander, S., Roberts, C. T., and Leroith, D., Growth inhibition of MCF7 breast cancer cells by stable expression of an insulin-like growth factor I receptor antisense ribonucleic acid. Endocrinology, *136:* 4298–4303, 1995.

12. Reiss, K., D'Ambrosio, C., Tu, X., Tu, C., and Baserga, R. Inhibition of tumor growth by a dominant negative mutant of the insulin-like growth factor I receptor with a bystander effect. Clin. Cancer Res., *4:* 2647–2655, 1998.

13. D'Ambrosio, C., Ferber, A., Resnicoff, M., and Baserga, R. A soluble insulin-like growth factor I receptor that induces apoptosis of tumor cells *in vivo* and inhibits tumorigenesis. Cancer Res., *56:* 4013–4020, 1996.

14. Prager, D., Li, H., Asa, S., and Melmed, S. Dominant negative inhibition of tumorigenesis *in vivo* by human insulin-like growth factor I receptor mutant. Proc. Natl. Acad. Sci. USA, *91:* 2181–2185, 1994.

15. Arteaga, C. L., and Osborne, C. K. Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. Cancer Res., *49:* 6237–6241, 1989.

16. Arteaga, C. L., Kitten, L. J., Coronda, E. B., Jacobs, S., Kull, F. C., Allred, D. C., and Osborne, C. K. Blockage of type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J. Clin. Investig., *84:* 1418–1423, 1989.

17. Gansler, T., Furlanetto, R., Gramling, T. S., Robinson, K. A., Blocker, N., Buse, M. G., Sens, D. A., and Garvin, A. J. Antibody to type I insulin-like growth factor receptor inhibits growth of Wilms' tumor in culture and in athymic mice. Am. J. Pathol., *135:* 961–966, 1989.

18. Liu, X., Turbyville, T., Fritz, A., and Whitesell, L. Inhibition of insulin-like growth factor I receptor expression in neuroblastoma cells induces the regression of established tumors in mice. Cancer Res., *58:* 5432–5438, 1998.

19. Corsello, S. M., Rota, C. A., Putignano, P., Della Casa, S., Barnabei, A., Migneco, M. G., Vangeli, V., Barini, A., Mandala, M., Barone, C., and Barbarino, A. Effect of acute and chronic administration of tamoxifen on GH response to GHRH and on IGF-I serum levels in women with breast cancer. Eur. J. Endocrinol., *139:* 309–313, 1998.

20. Watton, S. J., and Downward, J. Akt/PKB localisation and 3′ phosphoinositide generation at sites of epithelial cell-matrix and cell-cell interaction Curr. Biol., *9:* 433–436, 1999.

21. Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B., and Cohen, P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B. Curr. Biol., *7:* 261–269, 1997.

22. Rotem-Yehudar, R., Galperin, E., and Horowitz, M. Association of insulin-like growth factor 1 receptor with EHD1 and SNAP29. J. Biol. Chem., *276:* 33054–33060, 2001.

23. Chow, J. C., Condorelli, G., and Smith, R. J. Insulin-like growth factor-I receptor internalization regulates signaling via the Shc/mitogen-activated protein kinase pathway, but not the insulin receptor substrate-1 pathway. J. Biol. Chem., *273:* 4672–4680, 1998.

24. Pandey, N. P., Nguyen, H. T., Sharma, G. D., Shi, S-J., and Kriegel, A. M. Ligand-regulated internalization, trafficking, and down-regulation of guanylyl cyclase/atrial natriuretic peptide receptor-A in human embryonic kidney 293 cells. J. Bio. Chem., *277:* 4618–4627, 2002.

25. Drebin, J. A., Link, V. C., Stern, D. F., Weinberg, R. A., and Green, M. I. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. Cell, *41:* 695–706, 1985.

26. Shepard, H. M., Lewis, G. D., Sarup, J. C., Fendly, B. M., Maneval, D., Mordenti, J., Figari, I., Kotts, C. E., Palladino, M. A., Ullrich, A., and Slamon, D. Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic. J. Clin. Immunol., *11:* 117–127, 1991.

27. Fan, Z., Lu, Y., Wu, X., and Mendelsohn, J. Antibody-induced epidermal growth factor receptor dimerization mediates inhibition of autocrine proliferation of A431 squamous carcinoma cells. J. Biol. Chem., *269:* 27595–27602, 1994.

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