Original Article

Mechanisms of antibody-mediated insulin-like growth factor I receptor (IGF-IR) down-regulation in MCF-7 breast cancer cells

Masahiro Ohtani, Maki Numazaki, Yukiko Yajima, Yoko Fujita-Yamaguchi*

Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, Japan.

Abbreviated Names:

Ohtani M, Numazaki M, Yajima Y, Fujita-Yamaguchi Y

***Address Correspondence to:**

Dr. Yoko Fujita-Yamaguchi, Department of Applied Biochemistry, Tokai University School of Engineering, 1117 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan.

e-mail: xxxxxx

Tel: xxxxxx

Fax: xxxxxx

Conflict of Interest: ……

Abstract

The insulin-like growth factor I receptor (IGF-IR) plays a critical role in cell proliferation and survival. We previously reported that a recombinant anti-IGF-IR antibody, scFv-Fc, consisting of 1H7 monoclonal antibody (mAb)-derived single chain antibody (scFv) and human IgG_1 Fc, significantly suppressed breast tumor growth, and we proposed …...

Keywords: Receptor down-regulation, breast cancer, anti-IGF-I receptor antibodies, cancer therapy

1. Introduction

Insulin-like growth factors (IGFs) stimulate proliferation, motility, and survival of cells (*1*). The type I IGF receptor (IGF-IR) mediates the effects of IGF-I and -II. After molecular cloning of human IGF-IR in 1986 (*2*), ……

One of the authors previously reported the production of an anti-IGF-IR monoclonal antibody, 1H7 (*13*), and of the first recombinant anti-IGF-IR antibody consisting of the 1H7 single chain antibody (scFv) and human IgG_1 Fc domain (*14*,*15*). The scFv-Fc significantly suppressed breast tumor growth (*16-18*).…..

The details of IGF-IR down-regulation mechanisms by anti-IGF-IR antibodies are, however, not completely understood. The aim of this study was to determine mechanisms ……

2. Materials and Methods

2.1. *Materials*

IGF-I was purchased from Gro*Pep* (Adelaide, Australia). Anti-IGF-IR scFv-Fc was engineered and purified as described previously (*14*). Anti IGF-IR mAbs, 2C8 and 3B7, originally produced by the authors (*13*,*22*), as well as a polyclonal antibody against ubiquitin, 4PD1, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). ……

2.2. *Cell lines and culture*

MCF-7 cells, obtained from Dr. Douglas Yee of the University of Minnesota Cancer Center (Minneapolis, MN), were routinely maintained in Improved MEM with Zinc Option (Richter's modification) in ……

2.3. *Treatment of cells with IGF-I or mAb*

MCF-7 cells were grown in 3.5-cm dishes in regular growth media. Confluent cells (70%) were washed twice with PBS and serum deprived for 24 h in ……

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3. Results

3.1. *Characterization of intracellular signaling induced by IGF-I or various anti-IGF-IR antibodies*

Cellular proteins prepared from MCF-7 cells that had been treated with IGF-I or antibodies for 5 min were immunoblotted for ……

3.2. *Anti-IGF-IR antibody-induced IGF-IR down-regulation in MCF-7 cells*

MCF-7 cells, treated with either SFM (control) or SFM containing IGF-I, scFv-Fc, 1H7, 2C8, 3B7, 24-57, or αIR-3 for 24 h, were solubilized with TNESV lysis buffer…..

3.3. *Internalization of IGF-IR from clathrin-coated vesicles*

To determine whether IGF-IR is internalized from clathrin-coated vesicles or caveolae of the plasma membrane, IGF-IR down-regulation by scFv-Fc was……

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4. Discussion

The aim of this study was to determine whether or not anti-IGF-IR antibodies, with apparently distinct epitope specificities as summarized in Table 1, cause IGF-IR down-regulation, and if so, to determine the mechanisms by which these antibodies lead to internalization and degradation of IGF-IR. Effects of various anti-IGF-IR mAbs, 1H7, 2C8, 3B7, 24-57, and αIR3 along with scFv-Fc, on IGF-IR down-regulation were studied ……

As far as the effects of antibodies on IGF-IR signaling are concerned, scFv-Fc, 1H7, and 2C8 were agonistic. Although both scFv-Fc and 1H7 should have the same specificity since scFv-Fc is prepared from 1H7-producing hybridomas, the former had ……

It is clear that in MCF-7 cells, anti-IGF-IR antibody binding to the IGF-IR facilitated degradation of IGF-IR while IGF-I binding did not induce such receptor degradation. After internalization, IGF-IR can be either recycled back to the plasma membrane or processed for degradation into small pieces that ……

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In conclusion, more studies like this and others (*33*) are required to understand mechanisms of action by therapeutic anti-IGF-IR mAbs because at least 8 different anti-IGF-IR antibodies are……

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Table 1. Summary of characteristics of anti-IGFIR mAbs used in this study

mAb	Effect on IGF-IR signaling (This study)	Effect on IGF-I-binding	Epitope mapping on the α subunit of IGFIR
1H7	Stimulation	Inhibition (*13*)	440-514 (*29*)[a]
1H7 scFv-Fc	Stimulation	ND	440-514 (*29*)[a]
24-57	No effect	Inhibition (*23*)	440-514 (*30*)[a]
αIR-3	No effect	Inhibition (*24*)	223-274 (*31*)
3B7	No effect	Stimulation (*22*)	62-184 (*29*)
2C8	Stimulation	No effect (*13*)	ND

ND: Not determined; [a] Although 1H7 and 24-57 binding to the α subunit were competitive and the 440-514 domain was thus assigned as the epitope for both mAbs (*29*), this study suggested that their epitopes must differ (see Discussion).

Figure Legends

Figure 1. Comparison of intracellular signaling in MCF-7 cells after administration of various anti-IGF-IR antibodies. MCF-7 cells were grown in 3.5cm dishes in regular growth media. Confluent cells (70%) were washed twice with PBS and serum deprived for 24 h in SFM. Cells were ……

Figure 2. Anti-IGF-IR antibody induced IGF-IR down-regulation in MCF-7 cells. MCF-7 cells were either untreated (lane 1) or treated with ……

Figure 3. Internalization of IGF-IR from clathrin-coated vesicles. (A) MCF-7 cells preincubated with 2 mM methyl-beta-cyclodextrin (Mβ) or 7.5 μM chlorpromazine (CP) were treated …... **(B)** Shown are immunofluorescence images of MCF-7 cells after ……