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:

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

SUMMARY

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 …...

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 ……

……

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……

……

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 ……

……

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

References

1. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. Mol Pathol. 2001; 54:133-137. (As a sample of journal reference)

2. Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223. (As a sample of journal reference with more than 15 authors)

3. Shalev AY. Post-traumatic stress disorder: diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15. (As a sample of book reference)

4. Ministry of Health, Labour and Welfare of Japan. Dietary reference intakes for Japanese. *http://www.mhlw.go.jp/houdou/2004/11/h1122-2a.html* (accessed February 14, 2021). (As a sample of web reference)

……

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 ……