# Antibodies against human EPO Receptor

## Background of the Invention

Human erythropoietin (EPO) is a 166-aa glycoprotein which is involved in the proliferation and differentiation of erythroid progenitor cells. These cellular responses are mediated by the human EPO receptor (EPO-R), a 508-aa glycoprotein. Human EPO receptor (EPO-R) is a protein of 508 amino acid length (Swiss Prot P19235) containing a single transmembrane domain and has been classified as a member of the growth hormone subfamily of class I cytokine receptors. EPO-R is e. g. described in Winkelmann, J.C. et al., Blood 76 (1990) 24-30 and Jones, S.S. et al., Blood 76 (1990) 31-35. Activation of EPO-R occurs by dimerization (Matthews, D.J., PNAS 93 (1996) 9471-9476). EPO-R comprises eight cytoplasmic tyrosine sites which become phosphorylated upon stimulation with EPO (Li, K. et al., J. Biol. Chem. 278 (2003) 40702-40709; Wu, H.et al., Proc. Natl. Acad. Sci. USA 94 (1997) 1806-1810) resulting in "activated EPO-R".

Antibodies against EPO-R are e.g. known from Andrea, A.D., Blood 82 (1993) 46-52; Elliott, S., Blood 107 (2006) 1892-1895; Kirkeby, A., J. Nerosci. 164 (2007) 50-58; Miura, O., Arch. Biochem. 306 (1993) 200-208; and EP 1 146 056, EP 1 327 681, EP 0 773 962, EP 0 776 370, US 2002/0031806, US 2003/0215444, US 2004/0058393, US 2004/0071694, US 2004/0175379, US 2005/0227289, US 2005/0244409, US 2006/0018902, US 6,998,124, US 7,053,184, US 7,081,523, WO 1995/005469, WO 1996/003438, WO 2000/061637, WO 2004/035603 A2, WO 2005/100403 A2. However it is known form the state of the art, that known antibodies against EPO-R are not able to discriminate between non activated and activated EPO-R (see Jelkmann, W. et al., Crit. Rev. Onc/Hematol. 67 (2008) 39-61; Li, K. et al., J. Biol. Chem. 278 (2003) 40702-40709, and Wu, H. et al., Proc. Natl. Acad. Sci. USA 94 (1997) 1806-1810).

## Summary ~~of the Invention~~

The disclosure~~invention~~ comprises an antibody specifically binding to activated human EPO receptor and discriminating between non activated and activated EPO-R, which allows specific analysis of the activation of EPO-R especially in cells and biopsies from human tissue.

The disclosure~~invention~~ comprises an antibody, characterized in specifically binding human EPO receptor fragment TPPHLKYLYLVVSD (SEQ ID NO:25) comprising a phospho-tyrosyl residue at position 430 (underlined), GLSDGPYSNPYENSLIP (SEQ ID NO:26) comprising a phospho-tyrosyl residue at position 461 (underlined), or GLSDGPYSNPYENSLIP (SEQ ID NO:26) comprising a phospho-tyrosyl residue at position 465 (underlined).

Numbering relates to EPO receptor amino acid sequence of UniProtKB/Swiss-Prot P19235 without signal peptide.

In one aspect, there is provided an antibody binding to human EPO receptor, characterized in specifically binding EPO receptor fragment GLSDGPYSNPYENSLIP (SEQ ID NO:26) comprising a phospho-tyrosyl residue at position 465, whereby the antibody specifically binds to activated human EPO receptor and discriminates between non activated and activated EPO-R, the heavy chain variable domain comprises a CDR3 region of SEQ ID NO:17, a CDR2 region of SEQ ID NO:18 and a CDR1 region of SEQ ID NO:19 and the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO:21 and a CDR1 region of SEQ ID NO: 22, and whereby the antibody is specifically binding pY465 in ELISA at a S/N ratio of 10 or more at an antibody concentration of 0.1µg/ml.

An antibody according to the disclosure~~invention~~ does not bind EPO receptor fragment TPPHLKYLYLVVSD (SEQ ID NO:25) without a phospho-tyrosyl residue at position 430, GLSDGPYSNPYENSLIP (SEQ ID NO:26) without a phospho-tyrosyl residue at position 461, or GLSDGPYSNPYENSLIP (SEQ ID NO:26) without a phospho-tyrosyl residue at position 465.

An antibody according to the disclosure~~invention~~ specifically binds phosphorylated (activated) EPO receptor in lysates of UT7 cells which cells are expressing EPO-R in an amount of 100.000 to 500.000 receptors per cell (EPO-R expressing cells) and being treated with 500 pM EPO for activation. An antibody according to the invention does not bind EPO receptor in lysates of UT7 cells expressing EPO receptor and being not treated with EPO. Such binding can be measured by Western Blot.

Preferably the disclosure~~invention~~ comprises an antibody binding to human EPO-R, characterized in comprising as heavy chain variable domain CDR3 region a CDR3 region of SEQ ID NO: 1, 9 or 17.

Preferably the antibody is characterized in that the heavy chain variable domain comprises CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO:2 and a CDR1 region of SEQ ID NO:3 or CDR3 region of SEQ ID NO:9, a CDR2 region of SEQ ID NO:10 and a CDR1 region of SEQ ID NO:11 or CDR3 region of SEQ ID NO:17, a CDR2 region of SEQ ID NO:18 and a CDR1 region of SEQ ID NO:19.

Preferably the antibody is characterized in that the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO:2 and a CDR1 region of SEQ ID NO:3 and in that the light chain variable domain

comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5 and a CDR1 region of SEQ ID NO:6.

Preferably the antibody is characterized in that the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO:10 and a CDR1 region of SEQ ID NO:11 and in that the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO:13 and a CDR1 region of SEQ ID NO:14.

Preferably the antibody is characterized in that the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO:18 and a CDR1 region of SEQ ID NO:19 and in that the light chain variable domain comprises a CDR3 region of SEQ ID NO: 20, a CDR2 region of SEQ ID NO:21 and a CDR1 region of SEQ ID NO:22.

Preferably the antibody is characterized in that the heavy chain variable domain comprises SEQ ID NO:7, 15 or 23.

Preferably the antibody is characterized in that the light chain variable domain comprises SEQ ID NO:8, 16 or 24.

Preferably the antibody is characterized in that the heavy chain variable domain comprises SEQ ID NO:7 and the light chain variable domain comprises SEQ ID NO:8.

Preferably the antibody is characterized in that the heavy chain variable domain comprises SEQ ID NO:15 and the light chain variable domain comprises SEQ ID NO:16.

Preferably the antibody is characterized in that the heavy chain variable domain comprises SEQ ID NO:23 and the light chain variable domain comprises SEQ ID NO:24.

Preferably the antibody according to the disclosure~~invention~~ is characterized in binding to EPO-R with a binding affinity of at least $10^{-8}$ $M^{-1}$ to $10^{-12}$ $M^{-1}$ .

A further embodiment of the disclosure~~invention~~ is a nucleic acid encoding a heavy chain and a light chain of an antibody according to the disclosure~~invention~~.

Human and other constant chains are well known in the state of the art and e.g. described by Kabat (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218). It is further preferred that the antibody is of mouse origin and comprises the antibody variable sequence frame of a mouse antibody according to Kabat (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218).

The antibody according to the disclosure~~invention~~ is preferably of mouse, rabbit or human origin. As human antibody isotype IgG1 is preferred.

The disclosure~~invention~~ further provides expression vectors containing nucleic acid according to the disclosure~~invention~~ capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell, and host cells containing such vectors for the recombinant production of such an antibody.

The disclosure~~invention~~ further comprises a prokaryotic or eukaryotic host cell comprising a vector according to the disclosure~~invention~~.

The disclosure~~invention~~ further comprises a method for the production of a recombinant human or humanized antibody according to the disclosure~~invention~~, characterized by expressing a nucleic acid according to the disclosure~~invention~~ in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant. The disclosure~~invention~~ further comprises the antibody obtainable by such a recombinant method.

The disclosure~~invention~~ further comprises the use of an antibody according to the disclosure~~invention~~ to determine/detect mammalian cells bearing/expressing activated EPO receptor.

In another aspect, there is provided the use of an antibody as defined above for the *in vitro* determination of activated EPO receptor in human cells tissues, and biopsies.

Preferably an antibody according to the disclosure~~invention~~ is used to determine activated EPO receptor in lysates of biopsies of human tissue samples. Preferably such detection is performed by Western Blot.

**Detailed Description of the Invention**

The term "antibody" encompasses the various forms of antibody structures including but not being limited to whole antibodies and antibody fragments.

"Antibody fragments" comprise a portion of a full length antibody, preferably the variable domain thereof, or at least the antigen binding site thereof. Examples of antibody fragments include diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g.,