

[0046]

(2) Result

CALR proteins contained in the culture supernatants of the UT-7/TP0/CALR Del 52 and UT-7/TP0/CALR Ins 5 cells neoplastically proliferating, the UT-7/TP0/CALR (WT) cells as a control, and the UT-7/TP0/vec cells, which were prepared by transfecting human megakaryoblastic leukemia cell line UT-7/TP0 with a vector expressing a Del 52 or Ins 5 type mutant CALR protein, a vector expressing wild-type CALR, and an empty vector, respectively, were evaluated with an antibody that recognizes both the wild-type and the mutant-type (monoclonal antibody D3E6, Cell Signaling Technology, Inc., Cat# 12238) and a commercially available antibody that recognizes the mutant-type carboxyl-terminal sequence (monoclonal antibody CAL2, Dianova GmbH, Cat# DIA-CAL-250) (Figure 2). As a result, ~~as shown in Figure 2,~~ the immunoblotting using the antibody (B3 antibody) that recognizes the wild-type and mutant-type CALR proteins detected bands that cannot be recognized by the mutant-type antibody, in addition to the endogenous CALR, in the cells expressing the mutant CALR protein (Figure 3).

[0047]

Among peptide fragments obtained by fragmentation using NTCB at the amino-terminal side of the cysteine residue, the peptide fragments found only in the cleavage type of the mutant CALR protein (Del 52 type was used)

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CLAIMS

[Claim 1]

An antibody or a functional fragment thereof that binds to a cleaved mutant CALR protein, comprising an antigen-recognition site in (a) a polypeptide chain consisting of an amino acid sequence set forth in SEQ ID NO: 1 or (b) a polypeptide chain consisting of an amino acid sequence having deletion, substitution, or addition of one ~~to several~~ or two amino acids in SEQ ID NO: 1.

[Claim 2]

The antibody or the functional fragment thereof that binds to the cleaved mutant CALR protein according to Claim 1, wherein the antibody or the functional fragment thereof is selected from the following (c), (d), and (e):

(c) an antibody in which CDR1, CDR2, and CDR3 of the immunoglobulin VH chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 17, 18, and 19, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 17, 18, and 19;~~ and CDR1, CDR2, and CDR3 of the immunoglobulin VL chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 26, 27, and 28, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 26, 27, and 28;~~

(d) an antibody in which CDR1, CDR2, and CDR3 of the immunoglobulin VH chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 20, 21, and 22, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 20, 21, and 22;~~ and CDR1, CDR2, and CDR3 of the immunoglobulin VL chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 29, 30, and 31, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 29, 30, and 31;~~ and

(e) an antibody in which CDR1, CDR2, and CDR3 of the immunoglobulin VH chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 23, 24, and 25, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 23, 24, and 25;~~ and CDR1, CDR2, and CDR3 of the immunoglobulin VL chain are polypeptides consisting of the amino acid sequences set forth in SEQ ID NOs: 32, 33, and 34, respectively, ~~or amino acid sequences having deletion, substitution, or addition of one to several amino acids in the amino acid sequences set forth in SEQ ID NOs: 32, 33, and 34.~~

[Claim 3]

The antibody or the functional fragment thereof that binds to the cleaved mutant CALR protein according to Claim 1 or 2, wherein the antibody or the functional fragment thereof is selected from the following (C), (D), and (E):

(C) an antibody comprising an immunoglobulin VH chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having deletion, substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 5 and an immunoglobulin VL chain consisting of an amino acid sequence set forth in SEQ ID NO: 8 or an amino acid sequence having deletion, substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 8;

(D) an antibody comprising an immunoglobulin VH chain consisting of an amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having deletion, substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 6 and an immunoglobulin VL chain consisting of an amino acid sequence set forth in SEQ ID NO: 9 or an amino acid sequence having deletion, substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 9; and

(E) an antibody comprising an immunoglobulin VH chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 or an amino acid sequence having deletion,

substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 7 and an immunoglobulin VL chain consisting of an amino acid sequence set forth in SEQ ID NO: 10 or an amino acid sequence having deletion, substitution, or addition of one to ~~several~~ten amino acids in the amino acid sequence set forth in SEQ ID NO: 10.

[Claim 4]

An antibody or a functional fragment thereof that competes with the antibody according to Claim 2 or 3 for binding to an amino acid sequence portion set forth in SEQ ID NO: 1 in a cleavage mutant CALR protein.

[Claim 5]

A pharmaceutical composition comprising the antibody or the functional fragment thereof according to any one of Claims 1 to 4.

[Claim 6]

The pharmaceutical composition according to Claim 5, wherein the pharmaceutical composition is a diagnostic, preventive, or therapeutic agent for a myeloproliferative neoplasm.

[Claim 7]

A method for detecting a mutant CALR protein, comprising detecting the following polypeptide (a) or (b) in a biological sample:

(a) a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 1; or

(b) a polypeptide consisting of an amino acid sequence having deletion, substitution, or addition of one or two ~~to several~~ amino acids in SEQ ID NO: 1.

[Claim 8]

The method for detecting a mutant CALR protein according to Claim 7, wherein the detection of the polypeptide (a) or (b) is immunological detection using the antibody or the functional fragment thereof according to any one of Claims 1 to 4.

[Claim 9]

A method for screening for a myeloproliferative neoplasm therapeutic agent, comprising screening for a drug that recognizes the following polypeptide (a) or (b):

(a) a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 1; or

(b) a polypeptide consisting of an amino acid sequence having deletion, substitution, or addition of one or two ~~to several~~ amino acids in SEQ ID NO: 1.

[Claim 10]

A diagnostic method for a myeloproliferative neoplasm, comprising detecting the following polypeptide (a) or (b) in a biological sample:

(a) a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 1; or

(b) a polypeptide consisting of an amino acid sequence having deletion, substitution, or addition of one ~~to several~~ or two amino acids in SEQ ID NO: 1.

[Claim 11]

The diagnostic method according to Claim 10, wherein the detection of the polypeptide (a) or (b) is immunological detection using the antibody or the functional fragment thereof according to any one of Claims 1 to 4.

~~[Claim 12]~~

~~A preventive or therapeutic method for a myeloproliferative neoplasm, comprising administering the antibody or the functional fragment thereof according to any one of Claims 1 to 4.~~

[Claim 12~~13~~]

The antibody or the functional fragment thereof according to any one of Claims 1 to 4, for use in diagnosis of a myeloproliferative neoplasm.

[Claim 13~~14~~]

Use of the antibody or the functional fragment thereof according to any one of Claims 1 to 4, for producing a diagnostic agent for a myeloproliferative neoplasm.