PEPTIDES USED IN ANTI-CANCER PHARMACEUTICAL COMPOSITION SCREENING METHOD AND THE APPLICATION THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application claims the priority benefit of Taiwan patent application number **102135112**, filed on September 27, 2013, and Taiwan patent application number **103127229**, filed on August 8, 2014.

FIELD OF THE INVENTION

[0001] The present invention relates to at least a polypeptide which could be used in screening for anti-cancer pharmaceutical composition and the application thereof, and more particularly to a polypeptide which could be used in screening for anti-APT binding-cassette transporter antibody and the application thereof.

BACKGROUND OF THE INVENTION

[0002] Chemotherapy is the main treatment method for middle-stage or terminal cancer. Even though chemotherapy kill most cancer cells in cancer patients, there are few, about 10^8~10^9 cancer cells, that do not be killed remain in the body of said patients. These remained cancer cells begin to express lots of ATP-binding-cassette transporter (Hereinafter referred to as "ABC transporter") and thereafter these ABC transporters are inserted into the plasma membrane of said remained cancer cells to pump most of the chemotherapeutic agents out of said cancer cells. That is, chemotherapeutic agents can not kill remained cancer cells anymore because remained cancer cells remove chemotherapeutic agents out via ABC transporter. This is known as cancer drug resistance and urgently needs method to kill these drug-resistant cancer cells.

[0003] Sarkadi et al. (Physiological reviews, Vol. 86 No. 4, 1179-1236, 2006) reported that drug-resistant relative ABC transporters are mainly the following subfamily：

1. ATP-binding cassette protein subfamily B： At least contain ABCB1 (also known as multi drug resistance protein 1, referred to as MDR1; and also known as permeability glycoprotein), ABCB4 (also known as MDR3), and ABCB11 (the sister of P-Glycoprotein).

2. ATP-binding cassette protein subfamily C： At least contains ABCC1 (also known as multi drug resistance related protein 1, referred to as MRP1), and ABCC2 (also known as multi drug resistance related protein 2, referred to as MRP2). Probably contain ABCC3~6, and ABCC10~11.

3. ATP-binding cassette protein subfamily G： At least contains ABCG2 (also known as mitoxantrone resistance protein, referred to as MXR; and also known as breast cancer resistance protein, referred to as BCRP).

[0004] Shyjan (US patent US7785812) discloses a method of precisely targeting and attacking drug-resistant cancer cells by recognizing ABC transporter, which is expressed abundantly on the surface of drug-resistant cancer cells. In US7785812 patent, a synthetic anti-ATP binding-cassette transporter antibody (Hereinafter referred to as "synthetic anti-ABC transporter antibody") conjugated with chemotherapeutic agents is provided (referred to as "antibody-conjugated drug"). Therefore, said antibody-conjugated drug recognizes and binds to drug-resistant cancer cells via said synthetic anti-ABC transporter antibody, and then kill said drug-resistant cancer cells via said conjugated chemotherapeutic agents.

[0005] However, the synthesis process of not only anti-ABC transporter antibody but also ABC transporter is not an easy matter. That is to say, correct folding of said synthetic antibody and said synthetic transporter is required, and thus there are always lots of difficulty and complex factors that cause an increase in cost. Moreover, the synthetic anti-ABC transporter antibody prepared according to the prior method may not proper for different kind of drug-resistant cancer cells because it recognizes just one kind of ABC transporter. In addition, it may cause severe kidney disease that use chemotherapeutic agents-conjugated anit-ABC transporter antibody for a long time.

SUMMARY OF THE INVENTION

[0006] In terms of the disadvantage of prior art, the present invention provides several easily prepared polypeptides rather than complex ABC transporters.

[0007] The present invention also provides several polypeptides which could be used to screen samples and then get a pharmaceutical composition containing multiple kinds of anti-ABC transporter antibodies, wherein said pharmaceutical composition could be used in different kinds of drug-resistant cancer cells.

[0008] The present invention further provides an anti-cancer pharmaceutical composition against drug-resistant cancer cells, wherein said anti-cancer pharmaceutical composition brings less burden to kidney and body.

[0009] The present invention also provides an anti-cancer pharmaceutical composition which enhances the cytotoxicity of natural killer cells in killing cancer cells.

[0010] The present invention further more provides an anti-cancer pharmaceutical composition which solves cancer drug resistance problem and synergizes with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

[0011] In accordance with an aspect of the present invention, there is provided a polypeptide, the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0012] Preferably, the polypeptide is capable of binding to at least one kind of anti-ATP binding-cassette transporter antibodies.

[0013] In accordance with another aspect of the present invention, there is provided a method of screening for anti-cancer pharmaceutical composition, containing using a polypeptide in said screening method; wherein the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0014] Preferably, the method contains using said polypeptide to screen human blood samples, human plasma samples, or human serum samples in order to get said anti-cancer pharmaceutical composition.

[0015] Preferably, the anti-cancer pharmaceutical composition contains at least one kind of anti-ATP binding-cassette transporter antibodies.

[0016] Preferably, the anti-cancer pharmaceutical composition could enhance cytotoxicity of natural killer cells.

[0017] Preferably, the anti-cancer pharmaceutical composition could synergize with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

[0018] In accordance with third aspect of the present invention, there is provided a purified or synthetic antibody capable of binding to a polypeptide; wherein the amino acid sequence of said polypeptide is SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0019] Preferably, the purified or synthetic antibody could enhance cytotoxicity of natural killer cells.

[0020] Preferably, the purified or synthetic antibody could synergize with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

[0021] In accordance with fourth aspect of the present invention, there is provided a detection kit for detecting the amount of anti-ATP binding-cassette transporter antibodies, wherein said detection kit contains a polypeptide, and the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0022] In accordance with fifth aspect of the present invention, there is provided a detection method comprising using a polypeptide-based probe to detect the amount of anti-ATP binding-cassette transporter antibodies in a sample, wherein said polypeptide-based probe contains a polypeptide, and the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0023] Preferably, the detection method for detecting the amount of anti-ATP binding-cassette transporter antibodies in a sample is based on enzyme-linked immunosorbent assay (ELISA), western blot, microarray,

bead-based purification and detection method, or other probe detection method.

[0024] Preferably, the detection method contains the following steps:

(a) coat a container with said polypeptide;

(b) remove a first remaining solution in said container;

(c) add a proper diluted sample of a sample to said container;

(d) remove a second remaining solution in said container;

(e) add a secondary antibody;

(f) remove a third remaining solution in said container;

(g) detect the amount of said secondary antibody in said container.

[0025] Preferably, said container is a 96-well plate.

[0026] Preferably, said sample is at least one of or the combination of a human blood sample, a human plasma sample, and a human serum sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] FIG. 1 is a flow chart presenting the method of detecting the amount of anti-ATP binding-cassette transporter antibodies according to the present invention.

[0028] FIG. 2 is a column chart presenting the effect of anti-cancer pharmaceutical plasma on cancer cell drug resistance.

[0029] FIG. 3 is a column chart presenting the effect of anti-cancer pharmaceutical plasma on cytotoxicity of natural killer cells.

[0030] FIG. 4 is a flow chart presenting the method of purification of anti- ATP binding-cassette transporter antibodies via the polypeptides provided by the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0031] For solving the drawbacks encountered from the prior art, the present invention provides several polypeptides which could be used in screening for anti-cancer pharmaceutical composition and the application thereof. Said polypeptides have a relatively simple structure and could be used in screening method to get pharmaceutical composition abundant in anti-ABC transporter antibody. Hence, said pharmaceutical composition could be used to kill drug-resistant cancer cells.

[0032] In human immune system, natural killer cells (normally be defined as the lymphocyte with $CD3^{-}CD56^{+}$ phenotype; hereinafter referred to as "NK cells") play an important role in killing cancer cells, wherein NK cells mainly kill cancer cells through antibody-dependent cell-mediated cytotoxicity (Hereinafter referred to as "ADCC"). If cancer cells express ABC transporter and hence become drug-resistant cancer cells, the endogenous or exogenous anti-ABC transporter antibody will bind to the ABC transporter. Therefore, NK cell targets and binds to the constant region of said anti-ABC transporter antibody via its CD16 molecule (The CD16 molecule on NK cell's surface also known as FcgRIII molecule, which is the receptor of Fc region of antibody and thus could bind to the constant region of antibody.) Consequently, NK cell-mediated antibody dependent cellular cytotoxicity (ADCC) would be induced by said anti-ABC transporter antibody and said NK cell would release IFN-γ to kill said drug-resistant cancer cells.

[0033] Base on inventor's experiences and study, the present invention provides a better method to kill drug-resistant cancer cells. Said method not only retains the advantage of using ABC transporter as target, but also removes the burden of preparing complex antibody/antigen and

chemical agent's side effect risk at the same time. That is, the present invention provides at least a linear polypeptide to screening for pharmaceutical composition containing multiple kinds of anti-ABC transporter antibodies. Said anti-ABC transporter antibodies could recognize and bind to ABC transporter on the surface of drug-resistant cancer cells and induce NK cell-mediated ADCC reaction to kill said cancer cells. Moreover, if said linear polypeptide is well designed, the anti-ABC transporter antibodies in said pharmaceutical composition may block ABC transporter at the same time and thus inhibit drug-resistant cancer cells from taking out chemotherapeutic agents. Therefore, the cancer drug resistance could be solved.

[0034] The following provides detailed instructions for the use of the embodiments of the present invention, and for the technology and features of the present invention. However, the embodiment is not intended to limit the present invention, and hence a person having ordinarily knowledge in the art may make various changes and modification included within the spirit and scope of the present invention.

[0035] Experiment 1：Design linear polypeptides of ABC transporters

Use bioinformatic method to survey regions in ABC transporters (the ones that lead to cancer drug resistance) to find out the regions with high affinity for human leukocyte antigen (referred to as HLA, which is human's major histocompatibility complex, or referred to as human's MHC). Linear polypeptides designed according to the amino acid sequence of these high affinity regions may have structures quite similar to the epitopes on ABC

transporter surface and thus could replace the ABC transporter to specifically binding to anti-ABC transporter antibodies.

[0036] After a series of screening and improvement, the present invention provides seven linear polypeptides of ABC transporters (generally, polypeptide is the peptide sequence consists of 20~50 amino acids, but not intended to limit the present invention), wherein the amino acid sequence of said seven linear polypeptides of ABC transporters are SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, and SEQ ID NO：7. Any one of these seven liner polypeptides of ABC transporters or their combination could bind to at least a kind of anti-ABC transporter antibody, and hence could be used in screening method to get anti-cancer pharmaceutical composition. Particularly, all of these seven liner polypeptides of ABC transporters or their combination have its own structure similar to at least one epitope of ABC transporters, wherein said ABC transporters specifically refers to the one cause cancer drug resistance. Therefore, these seven liner polypeptides of ABC transporters could bind to the antibody of said ABC transporters and thus could be used in screening method to get anti-cancer pharmaceutical composition which is abundant in anti-ABC transporter antibody.

[0037] The binding affinity between the liner polypeptides of ABC transporters and at least an anti-ABC transporter antibody is higher than the binding affinity between phosphate buffered saline (referred to as PBS; pH 7.4) and said anti-ABC transporter antibody.

[0038] Experiment 2：Prepare linear polypeptides of ABC transporters

Chemical method is used for the synthesis of said seven linear polypeptides of ABC transporter. Purity should be greater than or equal to 95%. Inventor entrusted Mission Biotech (a company providing custom peptide synthesis service located in Taipei, Taiwan; website: http://www.missionbio.com.tw/) to synthesize said seven linear polypeptides of ABC transporters.

[0039] Dissolve said synthesized linear polypeptides of ABC transporters in 67% acetic acid (5 mg linear polypeptides / 1 mL 67% acetic acid) separately and then store it at -20℃.

[0040] Experiment 3 ： Screen for anti-cancer pharmaceutical composition.

Mix at least a kind of said seven linear polypeptides of ABC transporters and a sample (for example, a human blood sample, a human plasma sample, or a human serum sample), and detect the amount of a polypeptide-antibody complex, the combination of linear polypeptides of ABC transporters and anti-ABC transporter antibody, in the mixture. If there are lots of polypeptide-antibody complex in the mixture, said sample is certainly abundant in anti-ABC transporter antibody and thus could be used as anti-cancer pharmaceutical composition to induce NK cell-mediated ADCC reaction. Therefore, the drug-resistant cancer cells expressing ABC transporter would be killed by said samples abundant in anti-ABC transporter antibodies.

[0041] Said seven linear polypeptides of ABC transporters could be used in the detection method to detect the amount of anti-ABC transporter

antibody in sample, wherein the detection method is Enzyme-linked immunosorbent assay (referred to as "ELISA") or other methods using polypeptides as probe, for example, western blot, microarray, or bead-based purification and detection method. Therefore, the samples which are abundant in anti-ABC transporter antibody can be found systematically. Preferably, the samples containing at least one kind of anti-ABC transporter antibody can be found and used as anti-cancer pharmaceutical composition, wherein said ABC transporter specifically refers to the one that leads to cancer drug resistance. Take ELISA for example, the detailed steps of detection and screening method are as follows.

[0042] Please see FIG. 1, which is a flow chart presenting the method of detecting the amount of anti-ATP binding-cassette transporter antibodies according to the present invention. The method of detecting the amount of anti-ATP binding-cassette transporter antibodies according to the present invention contains at least the following steps:

Step S1 : Coat a container with a mixture of the seven linear polypeptides of ABC transporters.

Step S2 : Remove a first remaining solution in the container.

Step S3 : Add a proper diluted sample of a sample to the container.

Step S4 : Remove a second remaining solution in the container.

Step S5 : Add a secondary antibody.

Step S6 : Remove a third remaining solution in the container.

Step S7 : Detect the amount of the secondary antibody in the container.

[0043] In Step S1, said container is a 96-well plate. In the detail of Step S1, add 100 μL said mixture of said seven linear polypeptides of

ABC transporters to a first well and a second well of said 96-well plate separately, add 100 μL linear polypeptide of a plant protein to a third well and a forth well of said 96-well plate separately, and then place said 96-well plate at 4℃ for 8 hours (or overnight).

[0044] Among them, both of the mixture of said seven linear polypeptides of ABC transporters and the linear polypeptide of said plant protein are diluted to the concentration of 5 ~ 20 μg/mL. In the mixture of said seven linear polypeptides of ABC transporters, the 7 linear polypeptides are mixed in the same proportion, wherein the amino acid sequence of said seven linear polypeptides are SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, and SEQ ID NO：7.

[0045] The first well coated with the mixture of said seven linear polypeptides of ABC transporters is referred to as treatment group. The third well coated with linear polypeptide of a plant protein is referred to as control group. The second well coated with the mixture of said seven linear polypeptides of ABC transporters is referred to as first blank control group. The forth well coated with linear polypeptide of a plant protein is referred to as second blank control group.

[0046] In Step S2, all of the first remaining solution in said first, second, third, and forth wells are removed. Preferably, said first, second, third, and forth wells are washed three times with phosphate buffered saline (pH 7.4).

[0047] In Step S3, said sample is a plasma sample separated from human body (it can be replaced by a blood sample or a blood serum separated from human body; however, the embodiment is not intended to limit the present invention). Said plasma sample is diluted 100 ~ 200 times

with phosphate buffered saline (pH 7.4) to obtain said proper diluted sample. In the detail of Step S3, add 100 μL said proper diluted sample of said sample to the first and third well separately, add 100 μL phosphate buffered saline (pH 7.4) to the second and forth well separately, and then place said 96-well plate at a suitable temperature for a period of time. Preferably, place said 96-well plate at room temperature for 2 hours.

[0048] In Step S4, all of the second remaining solution in the first, second, third, and forth wells are removed. Preferably, said first, second, third, and forth wells are washed three times with phosphate buffered saline (pH 7.4).

[0049] In Step S5, said secondary antibody specifically refers to the one that can specifically bind to human anti-ABC transporter antibody. For example, said secondary antibody is rabbit anti-human IgG (Fc specific) antibody or goat anti-human IgG (Fc specific) antibody. In the detail of Step S5, add 200 μL said secondary antibody which is diluted to a suitable concentration (for example, dilute 10000 times with phosphate buffered saline) to all of the wells, and place said 96-well plate at a suitable temperature for a period of time. Preferably, place said 96-well plate at room temperature for 2 hours.

[0050] In Step S6, all of the third remaining solution in said first, second, third, and forth wells are removed. Preferably, said first, second, third, and forth wells are washed 3~5 times with phosphate buffered saline (pH 7.4).

[0051] The detection principle of Step S7 is that transform a substrate to a colored product by an enzyme which is conjugated with said secondary antibody. The amount of the secondary antibody could be determined by detecting the amount of said colored product. Preferably, said enzyme

conjugated with said secondary antibody could transform tetramethylbenzidine (referred to as TMB) to a blue product. For the other example according the present invention, the secondary antibody is conjugated with a fluorescent substance, and the intensity of fluorescence can be used to determine the amount of the secondary antibody.

[0052] In the detail of Step S7, add 100 μL TMB to said first, second, third, and forth wells separately, wait for 20 minutes, and then add 50 μL 2M H2SO4 to stop the reaction. Detect the optical density value (Hereinafter referred to as "OD value") of each well using measuring wavelength at 450 nm and reference wavelength at 630 nm. Finally, calculate the specific binding index (referred to as SBI) of said sample：

SBI = (OD value of treatment group − OD value of first blank control group) ÷ (OD value of control group − OD value of second blank control group)

[0053] Use SBI value as a reference to select samples (for example, a human blood sample, a human plasma sample, or a human serum sample). All the samples of which the SBI value is higher than 1.5 could be used as anti-cancer pharmaceutical composition and could induce NK cell-mediated ADCC reaction well. Among said anti-cancer pharmaceutical composition, the human blood sample with SBI value higher than 1.5 is referred to as anti-cancer pharmaceutical blood, the human plasma sample with SBI value higher than 1.5 is referred to as anti-cancer pharmaceutical plasma, and the human serum sample with SBI value higher than 1.5 is referred to as anti-cancer pharmaceutical serum.

[0054] Experiment 4：Test the efficacy of anti-cancer pharmaceutical composition

[0055] A. Prepare drug-resistant cancer cells

[0056] Seed MCF-7 breast cancer cells in a 24-well plate, wherein the initial concentration of MCF-7 breast cancer cell is 10^4 cells/mL. Add 100nM Mitoxantrone (the product name is Mitoxantrone dihydrochloride, from Sigma-Aldrich, America), which is a chemotherapeutic agents, and culture for a month, to the wells. All of the surviving cells are precisely the Mitoxantrone-resistant MCF-7 breast cancer cells.

[0057] B. Effect of anti-cancer pharmaceutical composition on cancer drug resistance

[0058] Seed Mitoxantrone-resistant MCF-7 breast cancer cells in 96-well plate at the density of 5000 cells per well. Add 100nM Mitoxantrone to control group wells and culture for a period of time. Add 100nM Mitoxantrone and 5% anti-cancer pharmaceutical plasma (add 5 μL anti-cancer pharmaceutical plasma to 95 μL medium) to treatment group wells and co-culture for a period of time. After culture for 24, 48, or 72 hours (1 day, 2 day, or 3 day), use MTT assay to measure cell viability.

[0059] Please see FIG. 2, which is a column chart presenting the effect of anti-cancer pharmaceutical plasma on cancer cell drug resistance. FIG. 2 presents control group (add Mitoxantrone) and treatment group (add Mitoxantrone and anti-cancer pharmaceutical plasma) on the horizontal axis from left to right in order. In control group, after culture for 1 day, 2 day, or 3 day, there is 6.4×10^3 cells/well, 9.4×10^3 cells/well, or 12.9×10^3 cells/well in order. In treatment group, after culture for 1 day, 2 day, or 3

day, there is 4.0×10^3 cells/well, 4.8×10^3 cells/well, or 6.2×10^3 cells/well in order. Among them, cells in treatment group are significantly fewer than cells in control group after culturing for 1 day, 2 day, or 3 day ($p<0.001$).

[0060] FIG. 2 indicates that anti-cancer pharmaceutical plasma inhibits the growth of Mitoxantrone-resistant MCF-7 breast cancer cells, and culture longer will lead to greater inhibitory effects. After co-culture with anti-cancer pharmaceutical plasma for 1, 2, or 3 day, cancer cell number decreases 37.5%, 48.9%, or 51.9% in order.

[0061] Based on these results, anti-ABC transporter antibodies in said anti-cancer pharmaceutical plasma can bind to and thus block ABC transporters of drug-resistant cancer cells, and thus prevent drug-resistant cancer cells from pumping the chemotherapeutic agent out of the cells. Therefore, the chemotherapeutic agent stays in the drug-resistant cancer cells and inhibits their growth continuously. That is, the 7 linear polypeptides of ABC transporters provided by the present invention could be successfully used in screening for anti-cancer pharmaceutical composition, wherein the anti-cancer pharmaceutical composition can solve the problem of cancer drug resistance through synergizing with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

[0062] C. Effect of anti-cancer pharmaceutical composition on cytotoxicity of natural killer cells

[0063] Seed Mitoxantrone-resistant MCF-7 breast cancer cells in 96-well plate at the density of 5000 cells per well. Add 2×10^4 natural killer cells to each of the control group well and culture for a period of time. Add 2×10^4 natural killer cells and 5% anti-cancer pharmaceutical plasma (add 5 μL anti-cancer pharmaceutical plasma to 95 μL medium) to each of the

treatment group well and culture for a period of time. After culture for 24, 48, or 72 hours (1 day, 2 day, or 3 day), use MTT assay to measure cell viability of Mitoxantrone-resistant MCF-7 breast cancer cells.

[0064] Please see FIG. 3, which is a column chart presenting the effect of anti-cancer pharmaceutical plasma on cytotoxicity of natural killer cells. FIG. 3 presents control group (add NK cells) and treatment group (add NK cells and anti-cancer pharmaceutical plasma) on the horizontal axis from left to right in order. In control group, after culture for 1 day, 2 day, or 3 day, there are 4.5×10^3 cancer cells/well, 5.9×10^3 cancer cells/well, and 8.5×10^3 cancer cells/well in order. In treatment group, after culture for 1 day, 2 day, or 3 day, there are 3.1×10^3 cancer cells/well, 3.5×10^3 cancer cells/well, and 5×10^3 cancer cells/well in order. Among them, cancer cells in treatment group are significantly fewer than cells in control group after culturing for 1 day, 2 day, or 3 day ($p<0.001$).

[0065] FIG. 3 indicates that anti-cancer pharmaceutical plasma could enhance cytotoxicity of natural killer cells, and culture longer will lead to higher cytotoxicity. The cytotoxicity of natural killer cells increases 31.1% on the first day (24 hours). The cytotoxicity of natural killer cells increases 40.7% on the second day (48 hours). The cytotoxicity of natural killer cells increases 41.2% on the third day (72 hours).

[0066] Based on these results, anti-ABC transporter antibodies in said anti-cancer pharmaceutical plasma can target drug-resistant cancer cells and induce NK cell-mediated ADCC reaction at the same time, and thus can specifically attack and kill drug-resistant cancer cells. That is, the 7 linear polypeptides of ABC transporters provided by the present invention could be successfully used in screening for anti-cancer pharmaceutical composition, wherein the anti-cancer pharmaceutical composition can

enhance cytotoxicity of natural killer cells to reduce the number of drug-resistant cancer cells.

[0067] Base on inventor's experiences and study, 150 ~ 300 mL anti-cancer pharmaceutical composition could be administered to animals/patients which/who need such treatment one time at each cycle of chemotherapy treatment.

[0068] Experiment 5 : Purification of anti-ABC transporter antibody

Please see FIG. 4, which is a flow chart presenting the method of purification of anti- ATP binding-cassette transporter antibodies via the polypeptides provided by the present invention.

Step S11 : Combine linear polypeptides of ABC transporters with magnetic beads to produce a polypeptide-bead conjugate.

Step S12 : The polypeptide-bead conjugate is mixed with a sample to make a mixture, wherein an antibody-polypeptide-bead complex is produced in the mixture.

Step S13 : Pass the mixture through a column which is placed in a magnetic field.

Step S14 : Pass a buffer through the column to separate anti-ABC transporter antibodies from the antibody-polypeptide-bead complex.

[0069] In the Step S11, the amino acid sequence of said polypeptides contains at least one of SEQ ID NO : 1, SEQ ID NO : 2, SEQ ID NO : 3, SEQ ID NO : 4, SEQ ID NO : 5, SEQ ID NO : 6, or SEQ ID NO : 7. Inventor entrusted Taiwan Advanced Nanotech (a company providing magnetic beads-based polypeptide service located in Taiwan; website: http://tanbead.com/) to synthesis said polypeptide-bead conjugate.

[0070] In Step S12, said antibody-polypeptide-bead complex is contained in said mixture, and said antibody is anti-ABC transporter antibody.

[0071] When performing Step S13, said antibody-polypeptide-bead complex in said mixture will be attracted by magnetic force and then be attached to said column.

[0072] In Step S14, said buffer specifically refers to the one that could break the linkage between said polypeptide-bead conjugate and said anti-ABC transporter antibody. In the case that Said polypeptide-bead conjugate is synthesized by the company providing magnetic beads-based polypeptide service, said company would provide the proper buffer.

[0073] The purified anti-ABC transporter antibody according to the present invention could induce NK cell-mediated ADCC reaction thus could be used to make anti-cancer drug or anti-cancer pharmaceutical composition.

[0074] That is, the present invention provides 7 kinds of linear polypeptide of ABC transporter, which could be used to screen human blood sample, human plasma sample, or human serum sample to get anti-cancer pharmaceutical composition (for example, to get anti-cancer pharmaceutical blood, anti-cancer pharmaceutical plasma, anti-cancer pharmaceutical serum, or purified anti-ABC transporter antibody). Said anti-cancer pharmaceutical composition not only enhances antitumor activities of natural killer cells to kill drug-resistant cancer cells, but also synergizes with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

[0075] Said linear polypeptide of ABC transporter provided by the present invention is the one of which the amino acid sequence is SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：

5, SEQ ID NO：6, or SEQ ID NO：7. Said linear polypeptide of ABC transporter provided by the present invention may also be the one consists of 20 ~ 50 amino acids, wherein the amino acid sequence of said linear polypeptide of ABC transporter contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

[0076] It could be comprehended that linear polypeptide derivatives of ABC transporter are also included within the spirit and scope of the appended claims, wherein the amino acid sequence of said linear polypeptide derivatives of ABC transporter contains at least one of the sequence obtained from adding, removing, or changing one or several amino acids of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7, wherein said polypeptide derivatives of ABC transporter could bind to at least one kind of anti-ABC transporter antibody.

[0077] While the invention has been described in terms of what is presently considered to be the most practical and preferred embodiments, it is to be understood that the invention needs not be limited to the disclosed embodiment. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and scope of the appended claims which are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures.

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<223> OTHER INFORMATION: A polypeptide designed from ATP-binding-cassette transporters.

<400> SEQUENCE: 7
Ala Val Gln Gly Val Ser Phe Lys Ile His Ala Gly Glu Thr Gly Ala
1 5 10 15
Gly Lys Ser Ser Leu Ala Ser Gly Leu Leu Arg Leu Gln Glu Ala
20 25 30

WHAT IS CLAIMED IS:

1. A polypeptide, the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

2. The polypeptide according to claim 1, wherein said polypeptide is capable of binding to at least one kind of anti-ATP binding-cassette transporter antibodies.

3. A method of screening for anti-cancer pharmaceutical composition, containing using a polypeptide in said screening method; wherein the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

4. The method according to claim 3, containing using said polypeptide to screen human blood samples, human plasma samples, or human serum samples in order to get said anti-cancer pharmaceutical composition.

5. The method according to claim 3, wherein said anti-cancer pharmaceutical composition contains at least one kind of anti-ATP binding-cassette transporter antibodies.

6. The method according to claim 5, wherein said anti-cancer pharmaceutical composition could enhance cytotoxicity of natural killer cells.

7. The method according to claim 5, wherein said anti-cancer pharmaceutical composition could synergize with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

8. A purified or synthetic antibody capable of binding to a polypeptide; wherein the amino acid sequence of said polypeptide is SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID

NO：6, or SEQ ID NO：7.

9. The purified or synthetic antibody according to claim 8, wherein said antibody could enhance cytotoxicity of natural killer cells.

10. The purified or synthetic antibody according to claim 8, wherein said antibody could synergize with chemotherapeutic agents to reduce the number of drug-resistant cancer cells.

11. A detection kit for detecting the amount of anti-ATP binding-cassette transporter antibodies, wherein said detection kit contains a polypeptide, and the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

12. A detection method comprising using a polypeptide-based probe to detect the amount of anti-ATP binding-cassette transporter antibodies in a sample, wherein said polypeptide-based probe contains a polypeptide, and the amino acid sequence of said polypeptide contains at least one of SEQ ID NO：1, SEQ ID NO：2, SEQ ID NO：3, SEQ ID NO：4, SEQ ID NO：5, SEQ ID NO：6, or SEQ ID NO：7.

13. The detection method according to claim 12, wherein said detection method for detecting the amount of anti-ATP binding-cassette transporter antibodies in a sample is based on enzyme-linked immunosorbent assay (ELISA), western blot, microarray, bead-based purification and detection method, or other probe detection method.

14. The detection method according to claim 12, containing the following steps:

(a) coat a container with said polypeptide;

(b) remove a first remaining solution in said container;

(c) add a proper diluted sample of a sample to said container;

(d) remove a second remaining solution in said container;

(e) add a secondary antibody;

(f) remove a third remaining solution in said container;

(g) detect the amount of said secondary antibody in said container.

15. The detection method according to claim 14, wherein said container is a 96-well plate.

16. The detection method according to claim 12, wherein said sample is at least one of or the combination of a human blood sample, a human plasma sample, and a human serum sample.