

ANTIBODY COMBINATION AND SYSTEM FOR DETECTING PROGNOSIS OF NASOPHARYNGEAL CANCER, AND USE

TECHNICAL FIELD

The invention belongs to the field of biomedicine, particularly it relates to an antibody combination, a system, and a use for predicting prognosis of nasopharyngeal carcinoma (NPC).

BACKGROUND

Nasopharyngeal carcinoma (NPC) is a unique type of head and neck tumor, particularly prevalent in south China and often referred to as "Canton tumor". NPC originates in the nasopharynx with an insidious onset, and is typically diagnosed at intermediate or advanced stages.

The pathogenesis of NPC remains incompletely understood. Global studies have demonstrated a strong correlation between NPC and Epstein-Barr virus (EBV) infection. EBV infection is widespread in the population, with approximately 95% of adults globally carrying the virus. Numerous studies have shown EBV is linked to various cancers, including NPC, gastric carcinoma, and malignant lymphoma, leading the World Health Organization (WHO) to classify it as a Group 1 carcinogen. Most of NPC patients are EBV-positive. Following host infection, EBV enters latent phase and lytic replication phase. During latent phase, EBV predominantly expresses EBV-related nuclear antigens and latent membrane proteins (LMPs), while the lytic replication phase is characterized by the expression of viral capsid antigens (VCAs), early intracellular antigens, early membrane antigens, and late-related antigens. In NPC tumor tissues, EBV resides latently within tumor cells but may undergo reactivation into the lytic cycle from the latent phase, releasing abundant viral particles recognized by antibodies secreted by surrounding plasma cells. Thus, intratumoral antibodies in NPC not only identify viral particles to eliminate EBV-positive tumor cells, but also potentially predict disease status.

Traditional approaches detect antibody titers related to EBV in the peripheral blood of

NPC patients. However, such titers may not align with EBV activity in tumor tissues. Direct measurement of antibody titers within tumor tissues may accurately reflect disease status.

SUMMARY

To address the above technical defects, an objective of the present invention is to provide an antibody or an antibody combination for predicting prognosis of NPC.

Another objective of the present invention is to provide a system for predicting prognosis of NPC.

A further objective of the present invention is to provide use of the antibody combination for predicting prognosis of NPC.

To achieve these objectives, the invention provides an antibody combination for detecting prognosis of NPC, comprising at least one of immunoglobulin heavy constant mu (IGHM), immunoglobulin heavy constant delta (IGHD), immunoglobulin heavy constant alpha 1 (IGHA1), immunoglobulin heavy constant alpha 2 (IGHA2), immunoglobulin heavy constant gamma 1 (IGHG1), immunoglobulin heavy constant gamma 2 (IGHG2), immunoglobulin heavy constant gamma 3 (IGHG3), and immunoglobulin heavy constant gamma 4 (IGHG4).

These eight antibody components can be used individually or in any combination to predict prognosis of NPC.

In another aspect, the present invention further provides use of the antibody combination in the preparation of a reagent for determining prognosis of NPC.

In another aspect, the present invention further provides use of a product for quantifying the antibody combination in the preparation of the reagent for determining prognosis of NPC.

Preferably, the product for quantifying the antibody combination is a reagent for quantifying the mRNA or the protein of the antibody heavy chains. The product may also be any one of other reagents, chips, or tools for reflecting expression levels of marker mRNA or

protein.

In another aspect, the present invention provides a system for predicting prognosis of NPC, comprising:

an antibody heavy chain quantification arrangement for determining an expression level of heavy chain in the antibody combination of claim 1;

a data analysis arrangement for calculating based on the expression level of the antibody heavy chain and determining prognosis of NPC; and

a result output arrangement for outputting a prognosis result.

Preferably, the expression level may be determined by various methods, preferably the expression level processed by non-dimensional treatment, to further perform data analysis.

In some embodiments, the antibody heavy chain quantification arrangement comprises reagents, chips, detection tools, or kits for quantitatively detecting expression levels of marker mRNA or protein.

In some embodiments, the expression level of the antibody heavy chain is obtained by performing quantification of the expression level of mRNA or protein for the antibody heavy chain gene.

In some embodiments, the reagent for performing quantification of marker mRNA at the genetic level includes but is not limited to expression chips, mRNA sequencing tools, qPCR gene detection tools, and Nanostring technology gene detection tools. The reagent for performing quantification of marker protein at the protein level includes but is not limited to enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunohistochemistry, Western blot, electrophoresis, and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

In some embodiments, expression levels of mRNA or protein for each antibody

component are calculated and determined by performing quantification of expression levels of mRNA or protein of all characteristic genes for each antibody component, followed by performing single-sample Gene Set Enrichment Analysis (ssGSEA) on the expression levels of mRNA or protein of all characteristic genes for each antibody component. Then, the determined expression levels of mRNA or protein are normalized by different methods.

In some embodiments, if the expression level of IGHM is below 182.125, it is classified as high risk; if the expression level of IGHM is above 182.125, it is classified as low risk;

if the expression level of IGHD is below 2.985, it is classified as high risk; if the expression level of IGHD is above 2.985, it is classified as low risk;

if the expression level of IGHA1 is below 756.34, it is classified as high risk; if the expression level of IGHA1 is above 756.34, it is classified as low risk;

if the expression level of IGHA2 is below 560.23, it is classified as high risk; if the expression level of IGHA2 is above 560.23, it is classified as low risk;

if the expression level of IGHG1 is below 9570.435, it is classified as high risk; if the expression level of IGHG1 is above 9570.435, it is classified as low risk;

if the expression level of IGHG2 is below 560.23, it is classified as high risk; if the expression level of IGHG2 is above 560.23, it is classified as low risk;

if the expression level of IGHG3 is below 558.075, it is classified as high risk; if the expression level of IGHG3 is above 558.075, it is classified as low risk; and

if the expression level of IGHG4 is below 437.92, it is classified as high risk; if the expression level of IGHG4 is above 437.92, it is classified as low risk.

In some embodiments, other well-known methods may be used to determine specific risk thresholds.

By calculating the expression levels of the characteristic genes or proteins for the eight antibody heavy chains, the present invention enables predicting clinical prognosis for NPC patients, facilitating personalized treatment and improving survival.

BRIEF DESCRIPTION

Figure 1 depicts the expression levels of antibody heavy chains (IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4) in NPC patients grouped by high and low-viral-load groups based on EBV viral load in plasma (Figure 1A, upper panel), and the expression levels of antibody heavy chains (IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4) in NPC patients grouped by high and low-viral-load groups based on EBV viral load in tumor tissues (Figure 1B, lower panel).

Figure 2 depicts for progression-free survival (PFS) in NPC patients grouped as high and low-risk groups based on the expression levels of IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4, with Kaplan-Meier (KM) survival curves analysis revealing significant differences in PFS between the two groups, wherein the subpanels correspond to:(A) IGHM, (B) IGHD, (C) IGHA1, (D) IGHA2, (E) IGHG1, (F) IGHG2, (G) IGHG3, and (H) IGHG4.

DETAILED DESCRIPTION

To clarify the objectives, features, and advantages of the present invention, the following provides a detailed description with reference to the drawings and embodiments. It should be understood that the embodiments are merely illustrative and not limited to herein. Unless otherwise specified, sequencing and analysis methods used in the following embodiments are conventional.

The present invention will be further explained through specific experiments. It should be understood that the following content is provided solely to illustrate the present invention and should not be construed as limiting the protection scope of the present invention.

Embodiment

Pretreated nasopharyngeal tumor tissue samples were collected via nasopharyngeal biopsy from 59 newly diagnosed NPC patients at Sun Yat-sen University Cancer Center. mRNA transcriptome sequencing was performed for each sample to obtain gene expression profile for each sample. EBV copy numbers in peripheral blood and tumor tissues were measured for all 59 patients. Patients were classified into high and low-viral-load groups based on thresholds of 1000 copies/mL (plasma) and median values (tumor tissues).

Figure 1 shows the expression levels of IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4 antibody heavy chains in tumor tissues, grouped by high and low-viral-load groups in plasma (Figure 1A, upper panel) and tumor tissues (Figure 1B, lower panel). For each antibody, the left represents patients with high EBV viral load (≥ 1000 copies/mL), and the right represents patients with low EBV viral load (< 1000 copies/mL). The vertical axis shows Transcripts Per Million (TPM) normalized ($+0.001$) and log-transformed values.

Figure 1 demonstrates that in both plasma and tumor tissues of NPC patients, the expression levels of IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4 antibody heavy chains are significantly lower in patients with low EBV viral load compared to the patients with high EBV viral load ($p < 0.05$).

Combining published transcriptome data from 88 NPC patients (GSE102349), the expression levels of the eight antibody heavy chain genes were analyzed in 147 NPC patients (from Sun Yat-sen University Cancer Center). Receiver operating characteristic (ROC) curves were used to determine optimal thresholds for gene expression level of each antibody heavy chain. Patients were grouped into high and low expression groups for each antibody, and survival analysis was performed to compare PFS between these two groups. Survival curves were plotted using the Kaplan-Meier method, with statistical significance assessed using the Cox proportional hazards model.

The results in Figure 2 showed that NPC patients with high expression of any of the eight

antibody heavy chains exhibited better prognosis compared to the patients with low expression ($p < 0.05$).

These results indicate that the eight antibody heavy chains—IGHM, IGHD, IGHA1, IGHA2, IGHG1, IGHG2, IGHG3, and IGHG4—can serve as prognostic indicators for NPC patients, demonstrating significant prognostic value.

For risk stratification, the following criteria may be applied: if the expression level of IGHM is below 182.125, it is classified as high risk; if the expression level of IGHM is above 182.125, it is classified as low risk; if the expression level of IGHD is below 2.985, it is classified as high risk; if the expression level of IGHD is above 2.985, it is classified as low risk; if the expression level of IGHA1 is below 756.34, it is classified as high risk; if the expression level of IGHA1 is above 756.34, it is classified as low risk; if the expression level of IGHA2 is below 560.23, it is classified as high risk; if the expression level of IGHA2 is above 560.23, it is classified as low risk; if the expression level of IGHG1 is below 9570.435, it is classified as high risk; if the expression level of IGHG1 is above 9570.435, it is classified as low risk; if the expression level of IGHG2 is below 560.23, it is classified as high risk; if the expression level of IGHG2 is above 560.23, it is classified as low risk; if the expression level of IGHG3 is below 558.075, it is classified as high risk; if the expression level of IGHG3 is above 558.075, it is classified as low risk; and if the expression level of IGHG4 is below 437.92, it is classified as high risk; if the expression level of IGHG4 is above 437.92, it is classified as low risk.

Alternatively, other known methods may be used to determine specific risk thresholds.

The above embodiments are illustrative and do not limit the scope of the present invention. It should be understood that modifications or substitutions may be made to the details and forms of the technical solutions without departing from the spirit and scope of the present invention, and such modifications and substitutions fall within the protection scope of the present invention.

Claims

1. An antibody combination for detecting prognosis of Nasopharyngeal carcinoma, wherein the antibody combination comprises at least one of immunoglobulin heavy constant mu (IGHM), immunoglobulin heavy constant delta (IGHD), immunoglobulin heavy constant alpha 1 (IGHA1), immunoglobulin heavy constant alpha 2 (IGHA2), immunoglobulin heavy constant gamma 1 (IGHG1), immunoglobulin heavy constant gamma 2 (IGHG2), immunoglobulin heavy constant gamma 3 (IGHG3), and immunoglobulin heavy constant gamma 4 (IGHG4).
2. Use of the antibody combination of claim 1 in the preparation of a reagent for determining prognosis of Nasopharyngeal carcinoma.
3. Use of a product for quantifying the antibody combination of claim 1 in the preparation of the reagent for determining prognosis of Nasopharyngeal carcinoma.
4. The use of claim 3, wherein the product for quantifying the antibody combination is a reagent for quantifying the mRNA or the protein of antibody heavy chains.
5. A system for predicting prognosis of Nasopharyngeal carcinoma, comprising:
 - an antibody heavy chain quantification arrangement for determining an expression level of heavy chains in the antibody combination of claim 1;
 - a data analysis arrangement for calculating based on the expression level of the antibody heavy chain and determining prognosis of NPC; and
 - a result output arrangement for outputting a prognosis result.
6. The system of claim 5, wherein the antibody heavy chain quantification arrangement comprises reagents, chips, detection tools, or kits for quantitatively detecting expression levels of marker mRNA or protein.

7. The system of claim 6, wherein the expression level of the antibody heavy chain is obtained by performing quantification of the expression level of mRNA or protein for the antibody heavy chain gene.

8. The system of claim 6, wherein if the expression level of IGHM is below 182.125, it is classified as high risk; if the expression level of IGHM is above 182.125, it is classified as low risk; if the expression level of IGHD is below 2.985, it is classified as high risk; if the expression level of IGHD is above 2.985, it is classified as low risk; if the expression level of IGHA1 is below 756.34, it is classified as high risk; if the expression level of IGHA1 is above 756.34, it is classified as low risk; if the expression level of IGHA2 is below 560.23, it is classified as high risk; if the expression level of IGHA2 is above 560.23, it is classified as low risk; if the expression level of IGHG1 is below 9570.435, it is classified as high risk; if the expression level of IGHG1 is above 9570.435, it is classified as low risk; if the expression level of IGHG2 is below 560.23, it is classified as high risk; if the expression level of IGHG2 is above 560.23, it is classified as low risk; if the expression level of IGHG3 is below 558.075, it is classified as high risk; if the expression level of IGHG3 is above 558.075, it is classified as low risk; and if the expression level of IGHG4 is below 437.92, it is classified as high risk; if the expression level of IGHG4 is above 437.92, it is classified as low risk.