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Study and analysis of the use of bevacizumab in the treatment of breast cancer

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Abstract. This study aimed to explore the application of bevacizumab in breast cancer treatment. We reviewed the research progress and current status of bevacizumab, analyzed its mechanism of action, and introduced protein expression analysis to further investigate its therapeutic effect. Bevacizumab is an angiogenesis inhibitor that prevents tumor growth by binding to and blockingvascular endothelial growth factor (VEGF). Data were obtained derived from the United States Society of Clinical Oncology (ASCO), which employed reversed-phase protein array technology (RPPA) to analyze the expression levels of related proteins. This included data on 210 proteins from 55 CTx samples and 54 Bev + CTx samples. We conducted a follow-up analysis based on the existing studies of nine protein signatures with corresponding beta coefficients identified by Lasso regression. However, only the first eight proteins were analyzed due to insufficient data. Additionally, we analyzed four of the 210 proteins with high expression levels. Statistical analysis revealed differences in protein the expression levels between the CTx and Bev + CTx groups, though these differences were not statistically significant. Clustering results displayed protein expression data through heat maps, revealing a clustering relationship among several proteins. This study provides a reference for the application of bevacizumab in breast cancer treatment.

Keywords: Bevacizumab, Breast cancer, Reversed-phase protein array technology, Protein expression.

1. Introduction

In the United States, it is estimated that 30% of all new cancer cases in women in 2017 were breast cancer[1]. The female breast is composed of skin fibrous tissue, breast gland and fat. Breast cancer is a malignant tumor that occurs in the breast epithelial tissue. Although the breast is not an important organ to maintain human life activities, and in situ mammary cancer is not typically fatal, the early breast symptoms are mostly not subtle. These may include breast lumps, nipple discharge, axillary lymphadenopathy and other localized signs. Systemic symptoms such as loss of appetite, weight loss, and fatigue may also occur, Since breast cancer cells lack the characteristics of normal cells, their connections are weaker, allowing them to detach and spread through the blood or lymphatic system[2]. At present, breast cancer has become the most harmful disease to women's physical and mental health. Currently, more than 1.5 million women worldwide (25% of all women with cancer) are diagnosed with breast cancer each year. During cancer development, angiogenesis facilitates tumor growth by providing cancer cells with necessary nutrients and oxygen through blood supply[3]. In the 1980s, vascular endothelial growth factor was isolated, which is an important regulator of angiogenesis, and when vascular endothelial growth factor receptor (VEGF) is released, it stimulates the formation of cardiovascular disease, a process called angiogenesis. Inhibition of VEGF can prevent the growth of blood vessels around the tumor, thereby depriving the tumor of blood supply, so as to achieve anti-cancer effect[4].

VEGF consists of soluble glycoproteins linked by disulfide bonds, found in higher eukaryotes. VEGF regulates cell proliferation and migration, metabolic homeostasis, and tubulogenesis by binding to specific membrane-binding or co-receptors. In addition, VEGF tightly controls physiological angiogenesis and angiogenesis, affecting blood and lymphatic function in healthy or diseased adult tissues, as well as vascular permeability[5].

Bevacizumab, serving as the inaugural angiogenesis inhibitor crafted via this specific mechanism, is a recombinant humanized monoclonal antibody. Its mode of action involves binding to and obstructing VEGF, thus curbing tumor angiogenesis. In the year 2004, it received approval for the treatment of metastatic colorectal cancer and subsequently found its way into a diverse array of solid tumor indications on an international front. The initial phase I trial of bevacizumab got underway in 1997. This trial encompassed twenty-five patients afflicted with either measurable or assessable solid tumors. Among these patients were eight with sarcoma, seven with renal cell carcinoma, five with breast cancer, and two with lung cancer. The trial scrutinized five distinct dose levels, namely 0.1, 0.3, 1.0, 3.0, and 10 milligrams per kilogram. Bevacizumab was administered intravenously over a span of 90 minutes on days zero, twenty-eight, thirty-five, and forty-two. One patient grappling with renal cell carcinoma demonstrated a mild response, witnessing a reduction of roughly 25% in the sum of the vertical diameters of lung and lymph node metastases. Among the remaining patients, 48% achieved a stable disease condition. Bevacizumab showcases a linear pharmacokinetic profile and possesses a terminal half-life of 21 days. Subsequently, a phase Ib trial evaluated bevacizumab in combination with an assortment of standard cytotoxic agents. In this trial, bevacizumab was administered at a dose of 3 milligrams per kilogram on a weekly basis for eight weeks. The outcomes of this trial revealed that the addition of VEGF antibodies did not significantly augment the incidence of known cytotoxic adverse events[6]. Following this, a phase II trial was undertaken for five diseases, namely kidney cancer, non-small cell carcinoma, colorectal cancer, prostate cancer, and recurrent metastatic breast cancer. The experimental results indicated that there was no substantial increase in the toxicity of bevacizumab [6].

As an anti-tumor drug widely used in clinical practice, bevacizumab has become a key drug for the treatment of a variety of malignant tumors, including metastatic colorectal cancer, non-small cell lung cancer, glioblastoma, etc. In clinical practice, bevacizumab is often used in combination with chemotherapy, radiation therapy, or other targeted agents, which greatly improves survival and quality of life [7,8]. In terms of drug mechanism, the focus is on the ability to inhibit the biological activity of vascular endothelial growth factor. VEGF is a core factor that promotes tumor angiogenesis, and tumor growth and metastasis depend on new blood vessels to provide nutrients and oxygen. Bevacizumab inhibits tumor vascularization by specifically binding to VEGF to prevent it from interacting with receptors on the surface of endothelial cells. This not only limits the nutrient supply to the tumor, but also optimizes the tumor microenvironment and enhances the therapeutic effect of chemotherapy and radiotherapy. In addition, bevacizumab may also have functions such as regulating the immune system and acting on tumor cell proliferation and invasion, but its specific mechanism is still being further explored.

Currently, bevacizumab is used to treat metastatic colorectal cancer and advanced metastatic or recurrent non-small cell lung cancer. Serious adverse events associated with bevacizumab include gastrointestinal perforation, bleeding, and arterial thromboembolism, which are contraindicated during pregnancy. The introduction of bevacizumab has changed the treatment paradigm for a range of solid tumors, offering new therapeutic options and advancing the development of angiogenesis inhibitors[9]. Reverse Phase Protein Array (RPPA) is a high-throughput protein detection technology. It places different biological samples (e.g., cell lysates, tissue extracts, etc.) in a lattice form onto a solid support (e.g., nitrocellulose membrane) and then detects and quantifies them with antibodies against specific proteins. Compared to traditional protein detection methods, reversed-phase protein arrays offer the advantages of high throughput, low sample size, ability to detect large numbers of proteins simultaneously, and parallel comparison of protein expression levels in different samples. It has a wide range of applications in tumor research, drug discovery, and signaling pathway analysis [10].

This article focuses on bevacizumab research, utilizing public data to analyze the relationships between several protein properties through data analysis. The first chapter of this paper is an introduction, which mainly introduces the relevant background review; Chapter 2 is Data Analysis and Methods, which provides a detailed description of data sources and analysis methods. Chapter 3

presents the preliminary results of data analysis. Finally, the research is discussed and the corresponding conclusions are presentsed.

2. Data sources and methods

2.1. Data sources

The data of this paper are from the United States Society of Clinical Oncology (ASCO), but the views of this paper do not represent the views and opinions of the United States Society of Clinical Oncology (ASCO). The data in this paper included 55 samples (CTx 1 to CTx 55) and 54 additional samples (Bev + CTx 1 to Bev + CTx 54), each containing 210 proteins. In this paper, 12 proteins (ACC-pS79, Bcl2, Chk1, Fibronectin, Myosin_IIa_pS1943, NDRG1_pT346, Notch1, Syk, Collagen_VI, Myosin_11, PDGFR_beta, Stat5a) were selected for analysis.

To further advance our understanding of the anti-angiogenic NAT response, a protein expression profile was constructed in the NeoAva Phase II clinical trial. Sixty-seven and seventy-one patients with human epidermal growth factor receptor 2 (HER2)-negative, previously untreated, and 2.5 cm in ≥size were randomly assigned to receive CTx or Bev plus CTx, respectively, in the group of breast cancer patients. Within each treatment arm, 66 patients were included in the primary endpoint analysis (pCR). The 109 samples collected prior to treatment can be used for RPPA protein analysis in the CTx-treated group (N=55) and the Bev plus CTx-treated group (N=54). The expression levels of 12 proteins in CTx and Bev plus CTx were statistically analyzed. By using Lasso regression to couple protein expression to relative tumor size (post-NAT), a panel of 10 proteins with non-zero β coefficients was identified. A second round of iterative lassos was performed on a subset of these ten proteins, and the final intercepts and beta coefficients were given, one of which was reduced to zero, resulting in the final data for 9 proteins with corresponding beta coefficients. Since the ninth group of experimental data (TP53BP1) cannot be searched in the original experimental data, only the above eight experimental data (ACC-pS79, Bcl2, Chk1, Fibronectin, Myosin_IIa_pS1943, NDRG1_pT346, Notch1, Syk) are displayed in this article. Due to the lack of a ninth type of data (TP53BP1) in the database, four groups of proteins (Collagen_VI, Myosin_11, PDGFR_beta, Stat5a) were added for the analysis of this article. Most of the expression levels of the above four proteins in the database are higher than zero, and most of the expression levels of other proteins are less than zero, so the above four proteins are added to this article for analysis.

In conducting this study, our primary focus was on the role of bevacizumab in the treatment of breast cancer. In order to ensure the validity and reliability of the research results, we supported the research through some public data. Based on the protein expression reported in the previous literature and the need for our experimental design, we identified samples for the current study with the help of publicly available data.In practice, we faced incompleteness in publicly available data, but we expanded the sample size as much as possible and made sure that the sample contained the protein expression features required for our experimental purposes to improve the representativeness of the sample.

2.2. Admission criteria

To construct a profile that could predict response to bevacizumab plus CTx treatment, protein expression in patients with NAT (N = 54) with relative tumor size in pre-treatment biopsies was studied. Because low-variant proteins may not be highly predictive, clinical markers should have some degree of expression variability. To this end, the original group of 210 proteins was reduced by expression variance plots to present a mixed distribution, in which only proteins that predominantly belonged to higher variance were used in the adaptive Lasso regression model. By using Lasso regression, protein expression was combined with relative tumor size (post-NAT) to identify a group of ten proteins with non-zero beta coefficients. A second iteration of Lasso was performed on these ten protein subsets, resulting in the final intercept and beta coefficients, one of which was reduced to zero, resulting in nine protein signatures with corresponding beta coefficients [11]. Data for the ninth protein (TP53BP1) were missing from the dataset, so only the first eight proteins were analyzed. Due

to the lack of individual protein data, in order to analyze the comparison of the two sets of data more comprehensively, four proteins (Collagen_VI, Myosin_11, PDGFR_beta, Stat5a) with higher expression levels than other proteins were selected for analysis.

2.3. RPPA

Reverse Phase Protein Arrays (RPPAs) are a powerful proteomics research technique. Its development has benefited from the continuous advancement of biotechnology, especially in the detection and quantification of proteins. RPPA is capable of detecting multiple proteins in a large number of samples simultaneously with high sensitivity, specificity, and throughput. In terms of applications, RPPA is widely used in cancer research. It can be used to analyze the expression pattern of proteins in tumor tissues, help understand the mechanism of tumor initiation, development, and assess tumor response to treatment and prognosis. In addition, in drug development, RPPA helps to screen drug targets and evaluate the effect and potential side effects of drugs. In immunology research, it is possible to study changes in protein expression in immune cells and gain insight into the regulatory mechanisms of immune responses [12]. In conclusion, the development of reversed-phase protein array technology has provided a powerful tool for life science and medical research, facilitating a deep understanding of disease mechanisms and the development of new therapeutic strategies.

The reverse phase protein microarray (RPPA) embarked on an extensive and in-depth analysis of a grand total of 210 proteins that are closely associated with cancer. Among these numerous proteins, a considerable and significant number, precisely 54 to be exact, were discovered to be phosphorylated. The lysates derived from tumor proteins were subjected to a consecutive series of successive twofold dilutions, a meticulous process that was repeated a total of five times. This dilution range spanned from being entirely undiluted all the way to a ratio of 1:16. Antibodies were judiciously put to use for the specific purpose of probing, while the DAB colorimetric reaction was skillfully employed to bring about vivid visualization. The relative levels of protein in each individual sample were determined with utmost precision. All the data points were carefully normalized with explicit reference to the loading of protein. Additionally, all values were systematically transformed through log2 and precisely centered around the median for each distinct individual antibody.[11].

2.4. Cluster studies

Clustering is to divide a dataset into different classes or clusters according to a specific criterion, so that the similarity of data objects in the same cluster is as large as possible, and the difference of data objects in different clusters is as large as possible. A clustering heatmap: A charting tool that represents the degree of concentration in a data matrix or data in terms of changes in color. A darker color means a larger value, and a lighter color means a smaller value. It is often used to display data information on gene expression, protein interactions, metabolic pathway activity, etc.

Clustering heatmaps have the advantage of visualizing the distribution of data, as this tool maps each cell of the data matrix into a rectangle, and uses color to indicate the size or difference of the numeric value, visualizing the distribution and difference of data between samples and features. Secondly, it also has the feature of visual correlation, which can reveal the correlation of data by showing the correlation matrix between samples or features. The strength of the correlation can be expressed by how bright or dark the color is, which can help you find samples or features that are high or low in correlation. It can then also be used to discover patterns and clusters, discovering similarities and correlations between samples or features by clustering data "rows and columns". This method groups samples or features with similar features and helps identify patterns and categories in the data. Finally, he can present the annotation information to the reader by adding row and column annotation information, such as sample grouping, feature classification, sample traits, etc., and using different colors or labels to provide more detailed data interpretation.

2.5. Statistical method

In terms of data processing, the software used in this paper is Graph Prism. The independent samples t-test was used as the statistical method. First, the data divided into CTx and Bev plus CTx were imported into the software, and the data to be analyzed were selected to perform the normal test, if the data meet the normal distribution, the parameter test can be carried out, and if the data does not meet the normal distribution, the non-parameter test needs to be used. After analysis, this paper uses a non-parametric test, and then calculates the P value, if the P value is less than the preset significance level (0.05), it is considered that there is a significant difference in the overall mean of the two sets of data, otherwise the opposite is true.

In this paper, the following website was used for clustering:https://www.bioinformatics.com.cn/login/firstly, the existing data were characterized by normalization and dimensionality reduction. Feature selection is then carried out, and the most effective features are selected from the initial features and summarized in a table. Then, feature extraction is carried out, and new salient features are formed by transforming the selected features. Clusters were then clustered through the above websites, and clusters were obtained by measuring similarity based on protein expression levels. Finally, the clustering results were evaluated and analyzed.

3. Results

3.1. Statistical analysis of specific proteins

To truly deepen our comprehension of the anti-angiogenic NAT response, a protein expression profile was developed in the NeoAva Phase II clinical trial. Now, sixty-seven and seventy-one patients with human epidermal growth factor receptor 2 (HER2)-negative breast cancer who were previously untreated and had tumors of at least 2.5 cm in size were randomly allocated to receive either CTx or Bev plus CTx in the breast cancer patient cohort. Well, within each treatment group, 66 patients were included in the primary endpoint analysis (pCR). Moreover, the 109 samples collected before treatment can be employed for RPPA protein analysis in the CTx-treated group (N = 55) and the Bev plus CTx-treated group (N = 54). Then, the expression levels of 12 proteins in CTx and Bev plus CTx were statistically evaluated. By utilizing Lasso regression to link protein expression to relative tumor size (post-NAT), a set of 10 proteins with non-zero β coefficients was determined. Oh, a second round of iterative lassos was conducted on a subset of these ten proteins, and the final intercepts and beta coefficients were given. However, one of them was reduced to zero, leading to the final data for 9 proteins with corresponding beta coefficients. Since the ninth group of experimental data (TP53BP1) cannot be located in the original experimental data, only the aforementioned eight experimental data (ACC-pS79, Bcl2, Chk1, Fibronectin, Myosin_IIa_pS1943, NDRG1_pT346, Notch1, Syk) are showcased in this article. And the statistical results are presented in Figure 1.

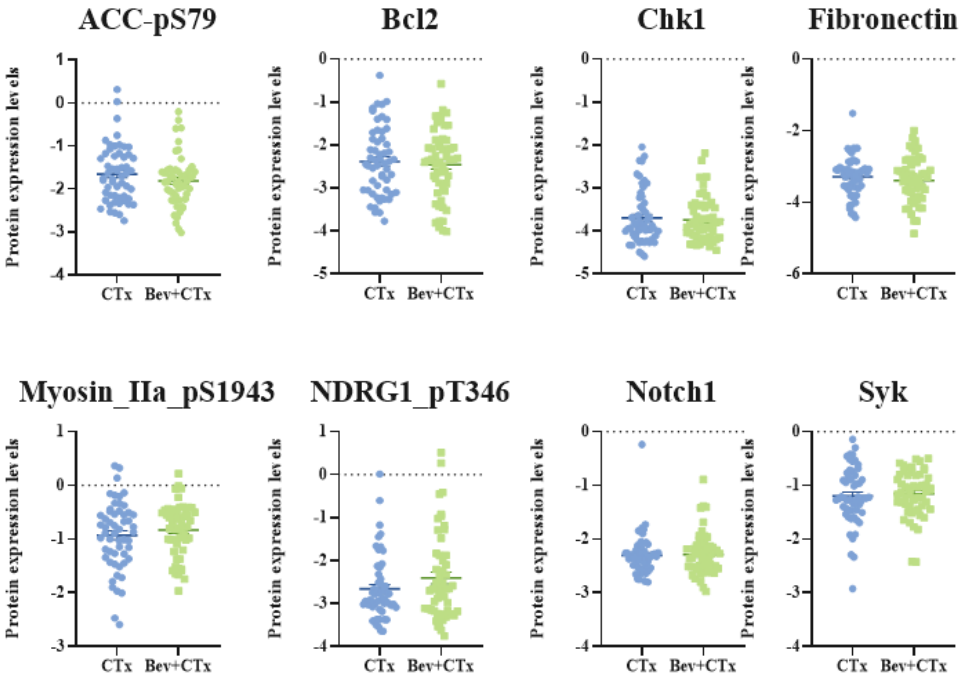

Figure.1 Punctate distribution of protein expression levels

The expression levels of the eight proteins under the conditions of CTx and Bev+CTX showed a certain degree of dispersion, and the expression levels of most samples were relatively concentrated, and it can be seen that the NDRG1_pT346 was the most widely dispersed, with the maximum value of 0.01 and the minimum value of -3.64 in the CTx group, and the maximum value of 0.51 and the minimum value of -3.75 in the Bev plus CTx group. However, no significant differences were shown overall. The mean and standard errors of other proteins are reflected in Table 1.

Table 1. Statistical table of protein expression level data

Protein	Constituencies	Mean	Standard error
ACC-pS79	CTx	-1.65	0.09
	Bev plus CTx	-1.81	0.08
Bcl2	CTx	-2.38	0.11
	Bev plus CTx	-2.46	0.11
Chk1	CTx	-2.46	0.08
	Bev plus CTx	-3.74	0.07
Fibronectin	CTx	-3.29	0.07
	Bev plus CTx	-3.39	0.09
Myosin_IIa_pS1943	CTx	-0.93	0.09
	Bev plus CTx	-0.83	0.07
NDRG1_pT346	CTx	-2.66	0.10
	Bev plus CTx	-2.41	0.14
Notch1	CTx	-2.30	0.05
	Bev plus CTx	-2.29	0.05
Syk	CTx	-1.20	0.07
	Bev plus CTx	-1.15	0.06

In Table 1, the mean and standard errors for the expression of the eight proteins are written in detail. The mean ranged from -3.5 to 0, with Myosin_IIa_pS1943 the largest in both the CTx and Bev plus CTx groups, at -0.93 and -0.83, respectively. In the CTx group, Fibronectin had the smallest mean protein expression (-3.29), and Chk1 (-3.74) in the Bev plus CTx group. The standard error generally ranged from 0 to 0.1, and only a few groups (Bcl2, NDRG1_pT346) had a standard error above 0.1. The expression of the first four histones (ACC-pS79, Bcl2, Chk1, Fibronectin) was higher in the CTx group, while the expression of the last four histones (Myosin_IIa_pS1943, NDRG1_pT346, Notch1, Syk) was higher in the Bev plus CTx group.

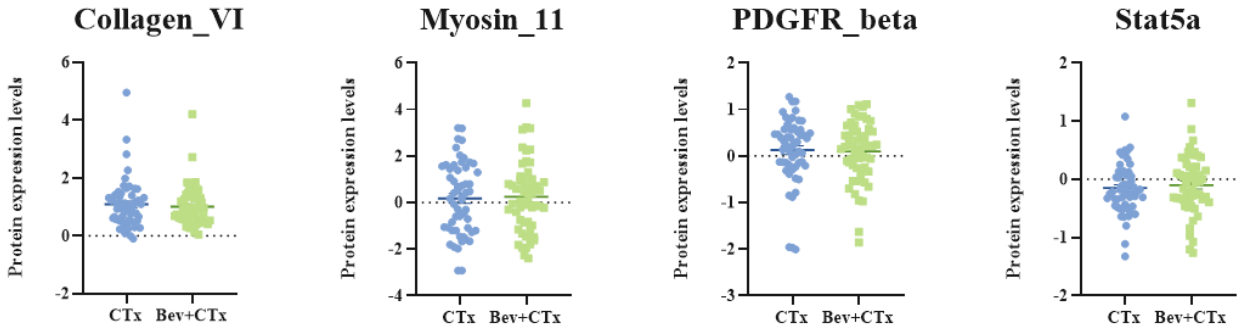

Figure.2 Dotted distribution of high protein expression levels

Due to the lack of a ninth type of data (TP53BP1) in the database, four groups of proteins (Collagen_VI, Myosin_11, PDGFR_beta, Stat5a) were added for the analysis of this article. Most of the expression levels of the above four proteins in the database are higher than zero, and most of the expression levels of other proteins are less than zero, so the above four proteins are added to this article for analysis.

The expression levels of Stat5a in the Collagen_VI, Myosin_11, PDGFR_beta, and Stat5a under CTx and Bev+CTX conditions showed a certain dispersion, and the protein expression levels of Collagen_VI were more concentrated than those of other proteins, with a mean value of 1.09 in the CTx group and 1.01 in the Bev plus CTx group. The expression levels of other proteins were more dispersed. For details, please refer to Table 2.

Table 2. Statistical table of high protein expression levels

Protein	Constituencies	Mean	Standard error
Collagen_VI	CTx	1.09	0.11
	Bev plus CTx	1.01	0.09
Myosin_11	CTx	0.18	0.21
	Bev plus CTx	0.24	0.21
PDGFR_beta	CTx	0.13	0.10
	Bev plus CTx	0.10	0.09
Stat5a	CTx	-0.15	0.06
	Bev plus CTx	-0.10	0.07

As can be seen from Table 2, except for Stat5a, the average expression level of other proteins is greater than zero, and the expression level of CTx group is higher than that of Bev plus CTx. Stat5a was the opposite of other proteins, the mean value of Stat5a was less than zero, and the protein expression level in the Bev plus CTx group was higher than that in the CTx group. In terms of standard

error, the Myosin_11 standard error was the largest and the only one of the twelve proteins included in the analysis with a value higher than 0.2.

3.2. Cluster analysis results

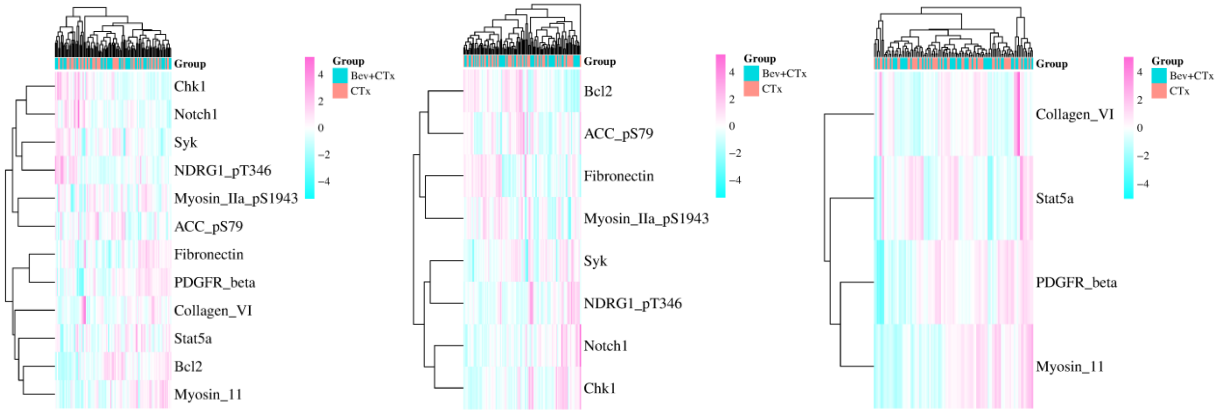

Figure.3 Protein expression cluster heat map

The clustering heatmap above represents the distribution of data for the expression of the selected proteins, with each column representing a sample, with the top row of orange representing the CTx group and green representing the Bev plus CTx group. One of the color blocks represents a piece of data, with a more pink color indicating a larger value, and a more cyan color bias indicating a smaller value. The first heat map shows the twelve proteins used in the analysis of the article, the second heat map shows the eight proteins screened out by the heat map, and the third heat map shows the four proteins whose protein expression levels are mostly above zero.

The first heat map shows a total of five groups of first layer clusters, in which these five groups are Chk1 and Notch1, Syk and NDRG1_pT346, Myosin_IIa_pS1943 and ACC-pS79, Fibronectin and PDGFR_beta, Bcl2 and Myosin_11. The second heat map shows a total of four groups of first-layer clusters, namely Bcl2 and ACC-pS79, Fibronectin and Myosin_IIa_pS1943, Syk and NDRG1_pT346, and Chk1 and Notch1. The third heatmap has only one set of first-layer clusters, with only PDGFR_beta and Myosin_11. The clustering of the above groups showed a scattered effect, that is, the expression levels of different proteins did not show a particularly significant relationship between the groups.

4. Conclusion

Bevacizumab, as an angiogenesis inhibitor, plays a crucial role in the treatment of breast cancer. This study provides an in-depth analysis of bevacizumab's mechanism of action and therapeutic effects, offering valuable insights for breast cancer treatment. The data of the study were sourced from the United States Society of Clinical Oncology (ASCO), ensuring the reliability and authority. The application of RPPA has made it possible to analyze 210 cancer-related proteins, offering comprehensive and detailed data support for research. This high-throughput detection technology allows simultaneous detection of multiple proteins across numerous samples with high sensitivity, and throughput. It facilitates insights into protein expression patterns in tumor tissues and the mechanism of tumor development.

In the statistical analysis of specific proteins, there were some differences in the expression levels of some proteins in the CTx and Bev + CTx groups, but the overall difference was not significant. Taking NDRG1_pT346 as an example, it has a maximum value of 0.01 and a minimum value of -3.64 in the CTx group, and a maximum value of 0.51 and a minimum value of -3.75 in the Bev plus CTx group, with the most widespread dispersion. Other proteins, such as ACC-pS79, Bcl2, Chk1, Fibronectin, etc., also showed some dispersion, but the expression levels of most samples were relatively concentrated. In addition, the expression of the first four histones (ACC-pS79, Bcl2, Chk1,

Fibronectin) was higher in the CTx group, while the expression of the last four histones (Myosin_IIa_pS1943, NDRG1_pT346, Notch1, Syk) was higher in the Bev + CTx group. At the same time, among the four proteins supplemented (Collagen_VI, Myosin_11, PDGFR_beta, and Stat5a), except for the mean value of Stat5a, which was less than zero and the protein expression level in the Bev + CTx group was higher than that in the CTx group, the mean expression levels of other proteins were greater than zero, and the expression level in the CTx group was higher than that in the Bev + CTx group. In terms of standard error, the Myosin_11 standard error was the largest and the only one of the twelve proteins included in the analysis with a value higher than 0.2, This phenomenon is caused by a large overall variance, i.e., large differences between individuals, from which the sample statistics drawn are also more discrete.suggesting that the expression level of the protein may vary greatly between samples.

The clustering results visually show the data distribution of protein expression through the clustering heat map. The first heat map shows a total of five groups of first-layer clusters of the twelve proteins, namely Chk1 and Notch1, Syk and NDRG1_pT346, Myosin_IIa_pS1943 and ACC-pS79, Fibronectin and PDGFR_beta, Bcl2 and Myosin_11, but the clustering of these groups showed a scattered effect, that is, the expression levels of different proteins did not show a particularly obvious relationship between groups. The eight proteins screened by the second heat map had four groups of first-layer clusters, namely Bcl2 and ACC-pS79, Fibronectin and Myosin_IIa_pS1943, Syk and NDRG1_pT346, Chk1 and Notch1, which also showed the characteristics of dispersion. The four proteins with protein expression levels mostly above zero in the third heat map have only one set of first layer clusters, PDGFR_beta and Myosin_11. These clustering results provide important clues for further analysis of the relationship and role between proteins, but also suggest that the interactions between proteins may be complex and require further in-depth study.

5. Discussion

There are some limitations to the study. Although RPPA technology has many advantages, it has high sample requirements and relatively high testing costs, which may limit its wide application in some clinical practices. In addition, due to the lack of data for the ninth protein (TP53BP1) in the dataset, only the first eight proteins were analyzed in this paper, which may lead to an incomplete understanding of protein characteristics and affect the completeness and accuracy of the study results.

Future research should focus on the following aspects. First, it is critical to expand the sample size, as more samples can provide more representative data, thereby improving the accuracy and reliability of the findings. Secondly, in-depth study of protein-protein interactions will help reveal the mechanism of tumor occurrence and development, and provide more precise targets for treatment. In addition, combined with other clinical indicators such as clinical symptoms, pathological features, treatment response, etc., comprehensive analysis can more comprehensively evaluate the treatment effect of bevacizumab and the prognosis of patients. Finally, it will be an important direction of future research to explore how to optimize the treatment plan, such as adjusting the drug dose and choosing the combination of drugs, so as to improve the efficacy of bevacizumab in the treatment of breast cancer and reduce the occurrence of adverse events. In general, this study provides a certain foundation for the application of bevacizumab in the treatment of breast cancer, but further in-depth research is still needed to verify and improve its mechanism of action and therapeutic effect, so as to provide more effective treatment strategies for breast cancer patients. The protein signatures identified in this study have some potential for predicting treatment response to bevacizumab plus CTx, but further studies are needed to verify their accuracy and reliability. Future studies could consider expanding sample sizes, delving into protein-protein interactions, and combining other clinical indicators to improve the accuracy of predictions. In addition, it is possible to explore how to optimize treatment regimens to improve the efficacy of bevacizumab in the treatment of breast cancer while reducing the occurrence of adverse events.

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