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Exosome molecular screening and modeling of its relationship with gene probes based on big data analysis

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Abstract As an important mediator of intercellular communication, this exosome plays a key role in tumor development. The purpose of this study is to screen exosome-related molecules in triple-negative breast cancer by bioinformatics methods, to explore their relationship with gene probes, and to construct a prognostic model. Methodologically, the gene expression profiles of 100 triple-negative breast cancer samples and 100 normal tissue samples were obtained from the TCGA database, combined with exosome signature-related genes from the ExoBCD database to screen for differentially expressed genes, and constructed a risk prediction model by LASSO regression and Cox regression analysis. The results showed that the prognostic model had high accuracy in the training set, with areas under the ROC curve of 0.8 at one year, 0.72 at three years, and 0.76 at five years. Univariate and multivariate Cox regression analyses demonstrated that the risk scores and the N stage could be used as independent prognostic indicators (P<0.001). In the external validation set, there was a significant difference in the overall survival of patients in the high and low risk groups. In this study, we successfully constructed a prognostic model for triple-negative breast cancer based on exosomal molecules, which provides new ideas for clinical risk assessment and individualized treatment.

Index Terms Exosome, Triple-negative breast cancer, Gene probe, Bioinformatics, Prognostic model, Tumor microenvironment

I. Introduction

A gene probe is a tool used to detect and analyze biomolecules such as DNA, RNA and proteins [1], [2]. By binding specifically to target molecules, gene probes can provide important information about the sequence, expression level and spatial location of target molecules, thus playing an irreplaceable role in biological and medical research [3]-[5]. With the continuous innovation and development of technology, the coordinated application of gene probes and exosome molecular screening technology has become is a biomedical research hotspot, and will also bring more breakthroughs and progress in biomedical research and clinical diagnosis and treatment [6]-[8].

In modern medical research, exosome molecular screening is a very important research direction [9]. However, the traditional exosome molecular screening methods have limitations in speed and accuracy. With the continuous progress of science and technology, big data analysis technology is not only widely used in the fields of finance, logistics, artificial intelligence, etc., but also has gradually been emphasized in the field of medical treatment, and exosome molecular screening technology based on big data analysis can effectively solve the above problems [10]-[13]. Exosomes are small membranous vesicles with a diameter of about 30 to 150 nm secreted by most of the cells in the body, which are widely present in various body fluids, and can carry and transmit important signaling molecules, thus affecting the physiological state of the cells and being related to the occurrence and progression of a variety of diseases [14]-[17]. Exosomes should be derived from intracellular lysosomal particles invaginated in the formation of multivesicular vesicles, which are released into the extracellular matrix after fusion of the outer membrane of multivesicular vesicles with the cell membrane [18]-[20]. Currently, while exosome molecular screening based on big data analysis is gradually transformed from experimental to clinical, and is widely used in lung cancer diagnosis and other aspects [21].

Exosomes are tiny vesicles with a diameter of 30-200 nm, which have been found to play an important role in intercellular communication in recent years, and are not only involved in physiological processes, but are also closely related to a variety of diseases, especially tumorigenesis and development. Triple-negative breast cancer (TNBC) is a highly invasive subtype of breast cancer that lacks estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, accounting for 15%-20% of the total number of breast cancers, but accounting for 83% of the morbidity and mortality rate, and is characterized by a young age of onset, a high degree of

invasiveness and a poor clinical prognosis. Tumor microenvironment plays a key role in malignant tumors and influences the process of tumor proliferation, invasion and metastasis. As a component of tumor-host interactions, exosomes are considered to be information-exclusive mediators in the tumor microenvironment. In this study, we intend to screen TNBC exosome-related prognostic genes by bioinformatics methods, obtaining gene expression data from TCGA and ExoBCD databases, and construct a prognostic risk model by combining with multifactor Cox regression analysis. Meanwhile, external validation was performed based on TNBC patient samples collected in our center to assess the clinical application value of the model. In addition, Nomograms were constructed by combining clinical factors to achieve individualized prediction of prognosis for TNBC patients. This study is expected to deepen the understanding of the mechanism of the role of exosomes in the development of TNBC and provide new ideas for the prevention, diagnosis and treatment of TNBC.

II. Exosomes and gene probes

II. A.Exosomes

Exosomes are tiny vesicles composed of a lipid bilayer membrane, produced by the endocytosis pathway and released by cytotoxicity, with diameters ranging from 30 to 200 nm and a spherical shape [22], [23].

Exosomes, as a type of extracellular vesicles (EVs), were initially thought to be waste disposal systems to secrete cellular metabolic wastes.

However, with the in-depth study of exosomes, it has been found that exosomes transfer functional proteins, metabolites, and nucleic acids to recipient cells and play an important role in intercellular communication.

II. A. 1) Source of exosomes

Exosomes from different cell sources are heterogeneous, mainly in terms of their function, composition, and immunogenicity. For example, functionally, exosomes derived from mesenchymal stem cells (MSCs) mainly play the roles of immunomodulation and promotion of tissue repair. Tumor cell-derived exosomes may promote tumor growth and metastasis. In contrast, macrophage-derived exosomes may affect the tumor microenvironment (TME) by regulating immune responses.

In terms of composition, MSCs-derived exosomes are enriched in a variety of extracellular matrix proteins, mRNAs, and multiple growth factors. Macrophage-derived exosomes, especially tumor-associated macrophages (TAMs), have high PD-L1 expression. Tumor cell-derived exosomes, on the other hand, may carry molecules that promote tumor growth and metastasis. In addition, in terms of immunogenicity, MSCs-derived exosomes have no tumorigenic risk and have lower immunogenicity. In contrast, exosomes from other cell sources, such as those from tumor cells, may carry tumor-associated antigens and have a certain degree of immunogenicity. Understanding the heterogeneity of exosomes from different cell sources can help to select appropriate vectors in disease treatment and drug development, as well as enable better evaluation of therapeutic effects.

(1) MSCs: MSCs, also known as pluripotent mesenchymal stromal cells, are present in almost all tissues of the human body, and the pluripotency of MSCs enables them to differentiate into a variety of cells such as adipocytes, chondrocytes, and osteoblasts. Because MSCs have the ability to stimulate tissue regeneration and regulate immune cells, they are widely used in the treatment of more and more diseases.

(2) Macrophages: Macrophages are immune cells, which are differentiated from monocytes through blood vessels, and can participate in both specific and non-specific immunity, and are the first line of defense for human immunity. Macrophages change their phenotype in response to environmental signals: M1 and M2 type polarized macrophages. Macrophage-derived exosomes differ from exosomes derived from other cells in several ways, and these differences are mainly in their composition, targeting, and biological functions.

(3) Tumor cells: Tumor cells are widely present in the parenchymal part of tumor tissues and are the main components of tumors. In order to adapt to the harsh environment, tumor cells rely on a variety of mechanisms to “survive”, including immune escape, epithelial mesenchymal transition (EMT), angiogenesis, autophagy, exosome release and so on. Among them, exosomes can maintain homeostasis, ensure cell survival, and ensure that the tumor microenvironment adapts to the rapidly growing tumor mass. Tumor cell-derived exosomes can enhance tumor growth, metastasis, and also promote tumor cell drug resistance.

II. A. 2) Biological functions of exosomes

Exosomes contain proteins, DNA, RNA, m RNA, mi RNA and other substances, so their biological functions are very diverse. The first recognized function of exosomes was to degrade certain proteins that are difficult for lysosomes to degrade as a complementary pathway to the lysosomal degradation pathway. The functions of exosomes vary depending on their cell of origin. For example, exosomes released by human natural killer (NK) cells express both NK cell markers and cytotoxic molecules such as perforin, which is also toxic to other cells and is active in inducing target cell death. It was found that neural stem cells attacked by inflammatory factors can produce

exosomes with interferon γ-binding interferon gamma receptor 1 (IFNGR1). Exosomes derived from human mesenchymal stem cells have been found to contain large amounts of m RNA, most of which are involved in cell differentiation, transcription, cell proliferation, and immunomodulation. Two of them were shown to be internalized and translated into full-length proteins in vitro and in vivo in murine renal epithelial cells, thus demonstrating the feasibility of horizontal transfer of m RNA.

DEK phosphoproteins were observed in exosomes derived from synovial macrophages, suggesting that exosomes are involved in joint inflammatory processes. The role of exosomes in the development of tumors and cancers has also been extensively studied, playing an important role in the tumor microenvironment and migration.

Numerous studies have shown the presence of exosomes carrying signaling and intracellular proteins in human cerebrospinal fluid, and it has been proposed that exosomes disrupt biological activity by neutralizing in vivo β amyloid plasticity primarily through the segregation of oligomers of their surface proteins. In the blood, exosomes enhance coagulation and are therefore involved in hemostasis. Exosomes derived from mononuclear blood cells have been reported to be involved in horizontal m RNA transfer and induce pro-angiogenic effects in vitro and in vivo, and leukocyte- and platelet-derived exosomes are thought to be responsible for the delivery of pro-angiogenic factors at the site of angiogenic sprouts.

II. A. 3) Exosome regulation of body cells

Exosomes are natural endogenous nanocarriers for drug delivery, which can deliver functional substances between different cells and play a major role as biomarkers of disease and effectors of physiopathological processes.

The multiple functions of exosomes depend on the type of cell of origin and include biological effects such as promotion of angiogenesis, inhibition of neuronal apoptosis, and anti-inflammation.

Studies have shown that exosomes secreted by vascular smooth muscle cells activate vascular repair and remodeling in response to external stimuli such as proinflammatory factors, and miR-143a contained within exosomes regulates vascular adhesive migration. The peripheral blood-derived exosome hyaluronan-binding protein 2 (Habp2) exacerbated neuroinflammatory injury in a mouse model of ischemic stroke by inhibiting astrocyte autophagy.

As a mediator of cellular communication, exosomes have the advantages of prolonged drug half-life, biocompatibility, nontoxicity, and penetration into deep tissues, which can replace adeno-associated viral vectors and nanoparticles for more efficient and safer drug-targeted delivery.

II. B.Gene Probes

Gene probe technology is also known as DNA probe technology, molecular hybridization.

Currently, gene detection means are divided into two categories: one is called heterophase hybridization technology or solid-phase hybridization technology (commonly Southern blotting, non-radioactive DNA probes, DNA biosensor probes, etc.). The other is called homophase hybridization or liquid phase hybridization techniques (commonly, two-probe conventional techniques, molecular beacon probes, etc.).

II. B. 1) Heterophase hybridization techniques

Heterophase hybridization, also known as solid-phase hybridization, is performed primarily in a solution environment, where one single strand of the DNA involved in the reaction is present in solution in a free form and the other single strand is immobilized on a solid-phase support (nitrocellulose filter membranes, nylon membranes, latex particles, magnetic beads, and microtiter plates, etc.). During the assay, the single chain bound to the substrate is immobilized by the solid phase support, and the single chain not bound to the substrate elutes in solution. Once the reaction product is solidified, it is not easily eluted, and is therefore most commonly used in food hygiene testing.

Heterophase hybridization by blotting is the earliest gene probe hybridization technique, first documented by homotopic labeling. As technology progressed, more food hygiene tests gradually adopted radioactive probes. The stronger the radioactivity intensity, the higher the positive detection rate and the better the test results.

Gene biosensor probe technology is a new tool based on gene probe technology and biosensor technology developed in recent years. The principle of gene biosensor probe technology is to fix the gene probe on the surface of the object, through the appropriate sensors and signal amplification devices, the reaction will be converted into quantifiable electrical, optical and other physical signals to enable food hygiene detection and monitoring.

II. B. 2) Homophilic hybridization techniques

Homophase hybridization technology, also known as liquid phase hybridization, does not require operations such as fixation of genes and deletion of unhybridized genes compared to heterophase hybridization. The two labeled probes of homophase hybridization mainly hybridize with the target gene in solution, and the hybrids are separated by equilibrium density gradient centrifugation according to the molecular weight to produce the assay results.

However, components that are not complementarily paired or failed after hybridization are mixed in the liquid phase, resulting in poor accuracy and precision of the final results. Molecular beacon probe technology is a hot topic in recent years, its main principle is fluorescence resonance energy transfer, fluorescence labeling at the end of the probe, only completely complementary genes can be hybridized with molecular beacon probe, which shows that molecular beacon probe technology has a very high specificity.

III. Prognostic modeling of breast cancer based on bioinformatics approaches

Breast cancer is the most common type of cancer among women worldwide. Despite significant advances in early detection and treatment, breast cancer remains the second leading cause of cancer-related deaths in women. Breast cancer can be categorized into triple negative breast cancer (TNBC) and non-TNBC based on histopathological classification.

TNBC is a highly aggressive subtype of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). It is most common in young and middle-aged women younger than 50 years of age and accounts for 15% to 20% of the total number of breast cancer diagnoses, but is responsible for 83% of breast cancer deaths. Compared with non-TNBC, TNBC is characterized by young age of onset, high invasiveness, poor clinical prognosis, high metastasis rate, and high recurrence rate.

In recent years, more and more evidence has shown that the tumor microenvironment plays a key role in malignant tumors, including tumor proliferation, invasion, metastasis, and tumor immunity to promote tumor progression and affect patient prognosis. Extracellular vesicles are recognized as new players in intercellular communication, and EVs are classified as exosomes, apoptotic vesicles, microvesicles, and other vesicles. Exosomes, as newly discovered components of tumor-host interactions, are increasingly widely recognized as information-exclusive mediators in the TME and are key entity molecules involved in the construction of the tumor microenvironment. Therefore, understanding the role and mechanism of exosomes in the development of TNBC is an important guide for the prevention, diagnosis and treatment of TNBC. Here in this paper, an attempt was made to analyze the role of exosomes in the development of TNBC using gene probe technology.

III. A. Materials and Methods

(1) Data sources

The Cancer Genome Atlas (TCGA) database serves as the largest cancer gene expression database. Its purpose is to deepen the understanding of the mechanisms of cancer occurrence and development by comprehensively analyzing genomic, transcriptomic, epigenetic, and clinical data of multiple cancers, thereby improving the prevention, diagnosis, and treatment of cancer.

(2) Materials

Breast cancer cell line MDA-MB-231 and fibroblast cell line CAF-95 were purchased from the Academy of Sciences cell bank.

Experimental consumables and reagents:

DMEM medium: Corning. Trypsin digestion solution: Bioengineering (Shanghai). Fetal Bovine Serum FBS: Ausbian Company, PBS buffer: prepared by Shanghai YiBeiRui Biomedical Technology Co. Serum-free cell freezing solution: Biosharp. DEPC water: Biosharp. Small interfering RNA: Shanghai Jimma Company. CCK8: Sigma Company.

GAPDH antibody: BBI Biologicals. ECH1 antibody: Abcam. C1R antibody: Abcam. MTRNR2L12 antibody: Abcam. SDS-PAGE kit: BBI Biologicals. BCA protein quantification kit: Hy Clone-Pierce. Prestained protein Prestained protein marker: Thermo Company. Chemiluminescent substrate: Millipore. Goat anti-rabbit fluorescent secondary antibody: BBI Biologicals.

Main experimental instruments

96-well plate: Corning, Cellometer Mini Cell Counter: Nexcelom. Fluorescence microscope: Olympus Corporation. Inverted microscope: Olympus Corporation; CO2 incubator: Thermo Corporation. Centrifuge: Thermo Fisher Scientific. Biological safety cabinet: Heal-Force. Voltage stabilizer: Shanghai Tannen Company. SDS-Acryl/Bis protein electrophoresis instrument: Shanghai Tannen Company. Protein transmembrane apparatus: Shanghai Tannen. 5417R benchtop refrigerated high-speed centrifuge: Eppendorf. Chemiluminescent Imaging System: GE. Hematocrit Plate: Seiko. Enzyme labeling instrument: Tecan infinite.

(3) Bioinformatics methods

In order to divide the samples in the dataset into a tumor segmentation, into a high-risk population with a higher level of disease threat, and another population with a relatively lower level of risk.

In order to select key genes with prognostic efficacy from multiple differential genes, Lasso and Cox regressions were performed with the “glmnet” and “survival” packages in R software to construct prognostic models. In the model, the samples were divided into high and low risk groups using the median as the cutoff.

Gene probe technology was utilized to screen for exosome-associated basic in triple-negative breast cancer.

III. B. Construction of a prognostic model for exosome-related genes in breast cancer

III. B. 1) Acquisition of data sets

The breast cancer dataset was obtained from the TCGA database, which included 1200 breast cancer samples and normal tissue samples. From them, 100 cases of triple-negative breast cancer samples and 100 cases of normal tissue samples were screened and gene expression profiles and clinical information were obtained.

A total of 100 genes related to exosome characterization were obtained from ExoBCD database download. The above information from public database sources was free from ethical conflict issues and ethical approval was not performed.

A total of 60 samples of TNBC patients attending the Department of Breast Surgery of the First Hospital of a province from January 2023 to December 2023 were collected, and whole RNA transcriptome sequencing was performed to obtain gene expression profiles. Detailed review of previous medical records was performed to obtain clinical information of the patients. Accurate patient survival information was obtained through outpatient review follow-up and telephone follow-up, and OS from diagnosis to the follow-up date was recorded.This article was ethically approved by the Ethics Committee of the First Hospital of the province, and all patients had signed informed consent.

III. B. 2) Analysis of differentially expressed genes

The TCGA dataset was analyzed for differences between the 100 TNBC tumor samples and 100 normal tissue samples using the “DESeq2” package in R. Genes with |log2 fold change|> 1 and corrected $P < 0.05$ were considered as differentially expressed genes in this paper.

The above differentially expressed genes were analyzed by one-way COX regression using the “survival” package in R. Genes with $P < 0.05$ were considered as prognostically relevant differentially expressed genes.

III. B. 3) Prognostic analysis of exosome-related genes

The 100 exosome-associated genes in the ExoBCD database and the above prognosis-associated differentially expressed genes were analyzed using the “ggvenn” package in R language, and plotted and visualized to identify TNBC exosome-associated prognostic genes.

III. B. 4) Gene enrichment analysis

Gene enrichment analysis is a bioinformatics-based method to identify the biological features and functions associated with specific genes. In order to evaluate the biological functions of exosome-associated prognostic genes, gene ontology (GO) enrichment analysis of exosome-associated prognostic genes was carried out using the “clusterProfiler” package in R. The GO terms included biological process (BP), molecular function (MF), and cellular component (CC). 0.05 for GO terms was considered significant.

III. B. 5) Construction and validation of a prognostic model for triple-negative breast cancer

The 100 cases of TNBC cohort in TCGA dataset were used as training set, and LASSO regression analysis of TNBC exosome-related prognostic genes was performed by using the R language “glmnet” package to reduce redundant genes and avoid model overfitting. The standardized regression coefficients were determined by multifactor COX regression analysis to establish the prognostic risk model.

Based on the median risk score of TNBC patients, patients were categorized into high-risk and low-risk groups. To assess the difference in survival between the two groups, Kaplan-Meier (K-M) survival curves were plotted using the “survival” package in R language. In addition, the “timeROC” package was used to generate ROC curves to predict the survival rates of TNBC patients at 1, 3, and 5 years to evaluate the predictive ability of the prognostic model.

The gene expression profiles and clinical information of 60 TNBC patients collected at our center were used as an external validation dataset, and the K-M survival curves were plotted to validate the accuracy of the model by dividing the patients into high- and low-risk groups according to their risk scores.

III. B. 6) Construction of Prognostic Nomograms for Breast Cancer

Nomograms, also known as column line plots, are a tool for visually displaying the results of multifactorial analyses. Column line plots are widely used in cancer prognostic studies, mainly because of their ability to reduce the

predictive model to a single numerical estimate that is specific to an individual patient's clinical characteristics and reflects the probability of an event occurring, which can be used to guide clinical decision making.

In order to further evaluate the predictive ability of the risk model, a multifactorial COX regression analysis was performed using the “survival” package in the R language, combining the risk scores and the patients' clinical data, including the T, N and M stages of the tumors, and the independent risk factors were screened out and used to construct the column charts to predict the 1-year, 3-year, and 5-year outcomes of the patients with TNBC. The independent risk factors were selected and used to construct a line graph to predict the OS of TNBC patients at 1, 3 and 5 years.

IV. Results

IV. A. Validation of the prognostic model

Two distinct groups of TNBC patients were delineated based on the median of their risk scores from the TCGA training set: one is a high-risk group with a high level of disease threat. The other is a population with a relatively low risk level.

The TCGA-KM curves are shown in Figure 1, and by looking at the KM curves, it can be seen that in the TCGA-TNBC dataset, patients in the high-risk category (100 cases in total), and in the low-risk category (also 100 cases) exhibit a significant disparity in overall survival, and this difference reaches a statistically significant level (p<0.0001).

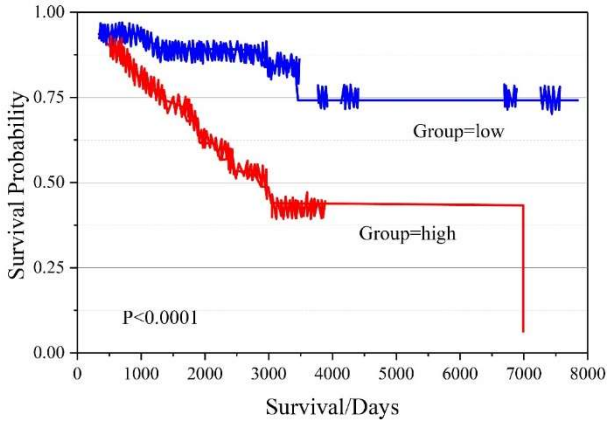

Figure 1: TCGA KM curve

The TCGA-ROC curve is shown in Fig. 2, and looking at the ROC curve again, it is found that the overall survival probabilities of one year, three years and five years correspond to an area under the ROC curve of 0.8, 0.72 and 0.76, respectively, for TNBC patients. This indicates that the model in this paper possesses a high degree of sensitivity and accuracy.

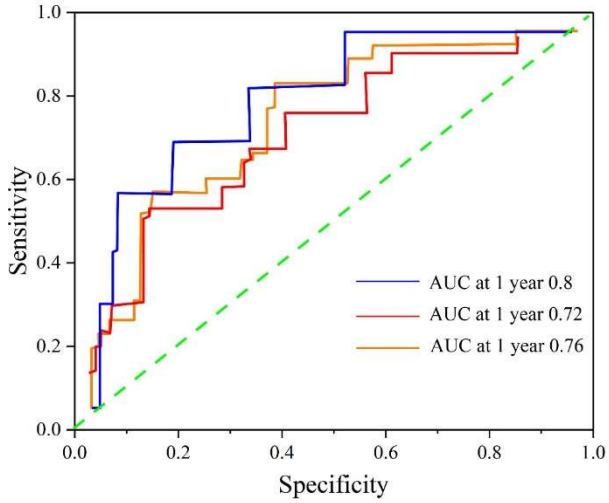

Figure 2: TCGA ROC curve

IV. B. Differential expression gene screening results

Gene expression data samples were collected through the TCGA database, which included 100 triple-negative breast cancer samples and 100 normal tissue samples of. These gene data samples were preprocessed and 2234 differential genes were obtained by $|logFC| > 1$ screening.

Among these genes, there were 859 and 1375 significantly up-regulated and down-regulated genes, respectively. The volcano plot of differentially expressed genes is shown in Figure 3. The horizontal coordinates represent the significance P-value, and the vertical coordinates represent the gene expression change times. The points in the graph are one-to-one correspondence with the genes. The blue and yellow points represent significantly down-regulated genes and up-regulated genes, respectively, and the gray points represent genes with insignificant expression changes.

Significantly down-regulated genes are mainly distributed near [-5,-0.5], genes with insignificant expression changes are mainly concentrated near 0, while significantly up-regulated genes are mainly concentrated at [0.5,5].

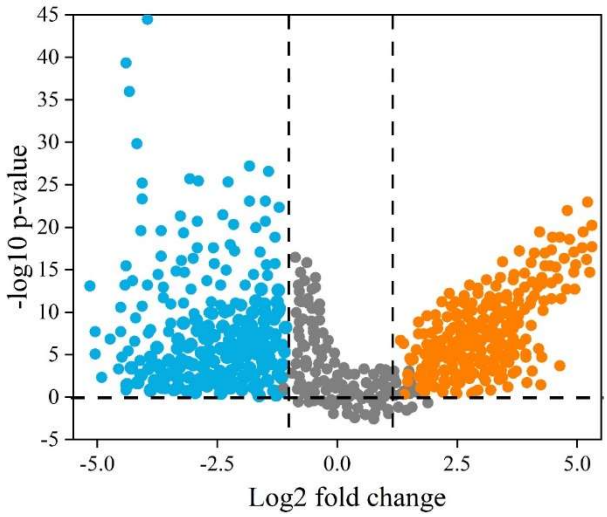

Figure 3: The difference expresses the gene volcano map

The principal component analysis (PCA) is shown in Figure 4. It confirmed that the tumor tissues and normal tissues were distributed in a discrete direction, and the light magenta and dark gray dots indicated normal tissues and tumor tissues, respectively. The number of tumor tissues was much more than that of normal tissues, and the distribution range was mainly concentrated in [-50,50].

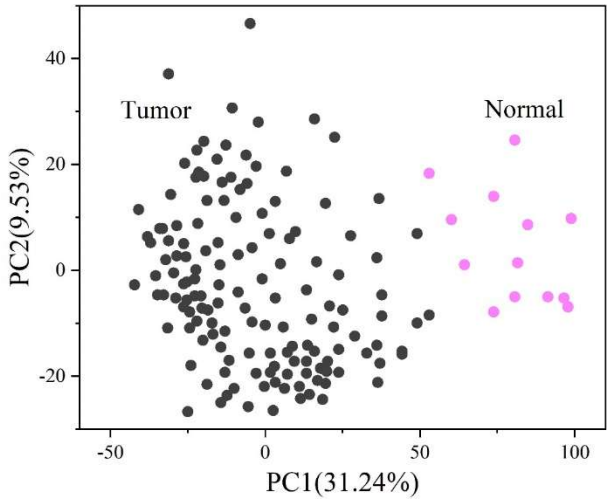

Figure 4: Major component analysis

IV. C. Univariate and multivariate COX regression analysis

Risk scores, age, T-stage, N-stage, M-stage, Ki-67, and clinical stage were subjected to univariate and multifactorial Cox regression analysis.

The unifactorial and multifactorial Cox statistics are shown in Table 1. The results showed that risk score, age, N staging, M staging, and risk score were able to serve as relevant risk factors. The N staging and risk score could even be used as independent prognostic indicators (P<0.001), demonstrating the reliability of the model.

Table 1: Single and multifactor cox statistics

Variable	Single factor COX			Multifactor COX		
	HR	95%CI	P VALUE	HR	95%CI	P VALUE
Age	1.012	0.962~1.005	0.515	1.021	0.968~1.025	0.226
M	5.074	1.550~16.014	0.010	4.895	0.691~29.341	0.192
N	2.893	1.839~3.855	<0.001	2.937	1.567~5.663	<0.001
T	1.131	0.788~1.425	0.963	0.704	0.421~1.439	0.404
Stage	1.224	0.956~1.327	0.143	1.229	0.461~3.784	0.625
Ki-67	0.986	0.921~1.008	0.152	1.001	0.978~1.056	0.712
Risk Score	1.305	1.181~1.334	<0.001	1.439	1.346~1.682	<0.001

V. Conclusion

In this study, we successfully constructed a prognostic model of triple-negative breast cancer based on exosomal molecules by bioinformatics method. Through the differential analysis of 100 TNBC samples and 100 normal tissue samples in the TCGA database, 2,234 differential genes were obtained by screening, of which 859 were up-regulated genes and 1,375 were down-regulated genes. The risk scoring system constructed using LASSO regression and multifactorial Cox regression analysis was able to effectively differentiate between the high- and low-risk patient groups, and the two groups showed significant differences in overall survival (P<0.001). The model demonstrated good accuracy in predicting 1-, 3- and 5-year survival in TNBC patients, with the area under the ROC curve reaching 0.8, 0.72 and 0.76, respectively. Univariate and multifactorial Cox regression analyses showed that the risk score (HR=1.439, 95% CI:1.346-1.682) and N staging (HR=2.937, 95% CI. 1.567-5.663) could be used as independent prognostic factors. In addition, by integrating risk scores and clinical factors, a nomogram was constructed to predict the prognosis of TNBC patients, which further improved the individualized precision of prediction. This study not only revealed the important role of exosome-related molecules in TNBC prognosis, but also provided new biomarkers and risk assessment tools for clinical risk assessment, therapeutic decision-making and prognosis prediction.

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