Genomic analyses identify marker molecules and processes in metastatic breast cancer tissues

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Abstract

Breast cancer metastasis is the major reason for deaths from breast cancer. Identification of breast cancer metastasis is of great importance for the management and prediction of cancer progression. However, the key genes and signaling pathways remain unclear in metastatic breast cancers. Our objective is to find the key molecules and signaling pathways by analyzing the RNA-sequence data. The GSE189411 was constructed by the Illumina NovaSeq 6000 (Mus musculus). The KEGG and GO analyses showed the cytokine–cytokine receptor interaction and human papillomavirus infection are the two major processes during the liver metastasis of breast cancer cells. Moreover, we discovered ten key relevant molecules including ITGB2, FCGR3A, CD86, CD80, FOXP3, SYK, CCR5, VCAM1, RAC2, ICAM1. Our study may provide novel insights for the early diagnosis of breast cancer metastasis.

Introduction

Breast cancer is the most malignancy and common death reason in women¹. Due to the early detection and systemic treatments, the mortality from breast cancer in the US has declined². However, breast cancer is still the most common reason for death in developing countries such as Africa and Asia³. Early breast cancer without metastases is a curable disease⁴. Primary surgery is not the optimal choice for all patients with breast cancer⁵. Though targeted therapies have increased the survival rate, the tumor relapses are caused by the drug resistance mechanisms⁶.

Breast cancer metastasis is characterized by local invasion and transferring cancer cells to other organs⁷. Evidence shows that metastasis can originate from genetic and epigenetic changes⁸. The genetic alterations occur in the DNA sites, but the epigenetic alterations are associated with DNA methylation and histone acetylation⁹.

In this study, we analyzed the metastatic tissues from breast cancer mouse models by using the RNA-seq data. We found a variety of DEGs and significant biological processes. We also performed the gene enrichment and constructed the protein-protein interaction (PPI) network and biological processes map to figure out the relationships among the DEGs. The DEGs and functional processes will help the early diagnosis of breast cancer metastasis.

Methods

Data resources

Gene dataset GSE189411 was downloaded from the GEO database. The data was produced by the Illumina NovaSeq 6000 (Mus musculus) (Guangxi medical university, Shuangyong Road, Nanning, China). The analyzed dataset includes 3 groups of controls and 3 groups of metastatic liver tissues.

Data acquisition and processing

The data were organized and analyzed by the R package as previously described¹⁰⁻¹⁴. We used a classical t-test to identify DEGs with $P < 0.05$ and fold change ≥ 1.5 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO)

KEGG and GO analyses were conducted by the R package (ClusterProfiler) and Reactome. $P < 0.05$ was considered statistically significant.

Protein-protein interaction (PPI) networks

The Molecular Complex Detection (MCODE) of Cytoscape software (US) was used to construct the PPI networks. The significant modules were produced from constructed PPI networks and String networks. The pathway analyses were performed by using Reactome (<https://reactome.org/>), and $P < 0.05$ was considered significant.

Results

Identification of DEGs in liver tissue after seeding the breast cancer cells

To identify the impacts of cancer cells' metastasis in the liver, we analyzed the RNA-seq data from the liver tissues with the transplantation of cancer cells in the axilla. A total of 925 genes were identified with the threshold of $P < 0.05$. The top up-and-down-regulated genes were shown by the heatmap and volcano plot (Figure 1). The top ten DEGs were selected in Table 1.

Enrichment analysis of DEGs in liver tissue after the transplantation of breast cancer cells

To further understand the potential mechanisms of breast cancer cell transplantation in the liver, we performed the KEGG and GO analyses (Figure 2). We identified the top ten KEGG signaling pathways including "Cytokine-cytokine receptor interaction", "Human papillomavirus infection", "Chemokine signaling pathway", "Axon guidance", "Lysosome", "Rheumatoid arthritis", "Osteoclast differentiation", "Viral protein interaction with cytokine and cytokine receptor", "Arrhythmogenic right ventricular cardiomyopathy", and "Glycosphingolipid biosynthesis – lacto and neolacto series". We identified the top ten biological processes of GO including "positive regulation of cell adhesion", "leukocyte migration", "cell-substrate adhesion", "cell chemotaxis", "anatomical structure homeostasis", "tissue homeostasis", "myeloid leukocyte migration", "granulocyte migration", "neutrophil migration", and "neutrophil chemotaxis". We identified the top ten cellular components of GO including "receptor complex", "collagen-containing extracellular matrix", "membrane raft", "membrane microdomain", "basal part of cell", "basal plasma membrane", "early endosome", "basolateral plasma membrane", "9+0 non-motile cilium", and "photoreceptor cell cilium". We also identified the top ten molecular functions of GO including "actin binding", "cell adhesion molecule binding", "G protein-coupled receptor binding", "actin filament binding", "immune receptor activity", "cargo receptor activity", "scavenger receptor activity", "G

protein-coupled chemoattractant receptor activity”, “chemokine receptor activity”, and “chemokine binding”.

PPI network analysis

To determine the relationships of the DEGs, we constructed the PPI network by using the 811 nodes and 2055 edges with Cytoscape software (combine score > 0.4). Table 2 showed the top ten interactive genes with the highest degree scores. The top two modules were indicated in Figure 3. We further analyzed the PPI and DEG network by Reactome map (Figure 4) and identified the top ten functional processes including "Chemokine receptors bind chemokines", "WNT ligand biogenesis and trafficking", "Interleukin-10 signaling", "YAP1- and WWTR1 (TAZ)-stimulated gene expression", "Rhesus blood group biosynthesis", "Common Pathway of Fibrin Clot Formation", "RND1 GTPase cycle", "Neutrophil degranulation", "Nef mediated downregulation of MHC class I complex cell surface expression", and "BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members" (Supplemental Table S1).

Discussion

The knowledge of the molecular mechanisms can improve the clinical treatment of breast cancers. Recent studies showed primary breast tumors that initiated metastases can be distinguished by their gene-expression profiles from those that remained localized¹⁵. Therefore, our study is to find out the potential marker genes in the metastasis tissues to improve the diagnosis of breast cancer in the early stage.

We figured out the “cytokine-cytokine receptor interaction” and “human papillomavirus infection” are the two major processes during the liver metastasis of breast cancer cells. Marcela Esquivel-Velázquez et al found a number of inflammatory cytokines such as IL6, IL17, and TNF are important for breast cancer initiation, promotion, angiogenesis, and metastasis¹⁶. Weilong Chen et al also found cytokines can change tumor cell behavior or reprogram tumor niche through several signaling pathways, thereby mediating the progress of drug resistance¹⁷. Niloofar Khodabandehlou et al found HPV DNA was detected in 48.6% of breast samples and HPV type 18 was the most prevalent virus genotype. Moreover, HPV was related to the upregulated inflammatory cytokines including IL1, IL6, and TNFα.

We also identified ten significant relevant molecules to the metastasis of breast cancer. ITGB2 is a prognostic marker gene for patients with breast cancer^{18, 19}. Patrick G Gavin et al found that the nucleotide polymorphisms in FCGR3A are strongly associated with breast cancer²⁰. Jun Fang et al found CD80 and CD86 are the prognostic markers of breast cancer²¹. Jiafeng Shou et al found the higher tumor infiltrating FOXP3+ Tregs is closely related to the worse outcome in breast cancer²². Circadian gene clocks and their target molecules regulate a variety of cell functions including cell metabolism, apoptosis, unfolded protein response, immune response, and aging²³⁻³⁴. Interestingly, FOXP3 was found to be controlled by the circadian clock in the T cell, which may further affect the microenvironment in cancers³⁵. SYK was found to possess the promotor and repressor activities of breast cancers. David J

Lamb et al found the SYK inhibitor BI1002494 showed no increased proliferation of breast cancer cells³⁶. Xuanmao Jiao et al found CCR5 is considered as a new therapeutic target for metastatic breast cancer³⁷. G protein-coupled receptor (GPCR) related signaling pathways involve different physiological and pathophysiological processes including metabolism, immune, and cancers³⁸⁻⁴⁹. Strikingly, CCR5 is a critical GPCR protein, which regulates several immune cells such as T-lymphocytes, macrophages, and dendritic cells⁵⁰. Xiaoqin Huang et al found patients with breast cancer and diabetes can promote cancer adhesion to vascular endothelium via ICAM1 and VCAM1⁵¹. Yi Zhang et al reported that RAC2 is the negative coefficient that was correlated with a better prognosis⁵².

In summary, our study identified the potential gene markers for the metastasis of breast cancer. The cytokine–cytokine receptor interaction and human papillomavirus infection are the mainly affected processes during the metastasis of breast cancer. This study may provide knowledge in the early diagnosis of metastatic breast cancer.

Declarations

Author Contributions

Xiuying Wang, Mengyao Wang: Methodology and Writing. Hanming Gu, James Liu: Conceptualization, Writing- Reviewing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

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Tables 1-2

Tables 1-2 are available in the Supplementary Files section.

Figures

Figure 1

Heatmap and volcano plot of DEGs in liver tissue after the transplantation of breast cancer cells

(A) Heatmap of significant DEGs. Significant DEGs ($P < 0.01$) were used to create the heatmap.

(B) Volcano plot for DEGs in liver tissue after the transplantation of breast cancer cells. The most significantly changed genes are highlighted by grey dots.

Figure 2

KEGG and GO analyses of DEGs in liver tissue after the transplantation of breast cancer cells

(A) KEGG analysis, (B) Biological processes, (C) Cellular components, (D) Molecular functions.

Figure 3

The PPI network analyses of DEGs in liver tissue after the transplantation of breast cancer cells

The cluster (A) and cluster (B) were constructed by MCODE.

Figure 4

Reactome map representation of the significant biological processes in liver tissue after the transplantation of breast cancer cells

Supplementary Files

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