GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Analysis of signaling pathways in recurrent breast cancer

J.Z. Wu[1], T.J. Yang[2], P. Lu[2] and W. Ma[3]

[1]Breast Cancer Diagnosis and Treatment Center Henan,
The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China
[2]Gastrointestinal Surgery Department, People's Hospital of Zhengzhou,
Zhengzhou, China
[3]Department of Oncology, First Affiliated Hospital of Zhengzhou University,
Zhengzhou, China

Corresponding author: W. Ma
E-mail: linmeiwangzz@hotmail.com

Genet. Mol. Res. 13 (4): 10097-10104 (2014)
Received August 5, 2013
Accepted September 22, 2014
Published December 4, 2014
DOI http://dx.doi.org/10.4238/2014.December.4.4

ABSTRACT. Breast cancer remains the second largest cause of death in women from cancer. By analyzing gene expression profiles in samples from breast cancer patients, 844 differentially expressed genes (DEGs) were identified in breast cancer metastasis. The 10 most significant signaling pathways identified through enrichment analysis contained DEGs were involved in oxidative phosphorylation, DNA replication, extracellular matrix-receptor interactions and others. Furthermore, survival analysis demonstrated that 5 of these signaling pathways were closely related to the survival time of breast cancer patients including basal transcription factors, cell cycle, ECM-receptor interaction, spliceosome, and DNA replication. Our findings increase the understanding of the network of signaling pathways involved in breast cancer metastasis and may provide theoretical support for further therapeutic study.

Key words: Breast cancer; Pathway; Survival analysis

INTRODUCTION

Breast cancer is considered the leading cause of cancer-related deaths in women worldwide (Kamangar et al., 2006). Treatment options for metastatic breast cancer have improved with the increase in knowledge regarding connections between signaling pathways and biological behaviors. However, several major clinical and scientific issues remain unresolved, including the prevention, diagnosis, tumor progression and recurrence, treatment, and therapeutic resistance of breast cancer. There are 5 major molecular subtypes of breast cancer: basal-like, luminal A, luminal B, HER2+/ER-, and normal breast-like (Perou et al., 2000; Hu et al., 2006; Sørlie et al., 2006), which are conserved in different ethnic populations (Yu et al., 2004). Breast cancer is highly heterogeneous at both the molecular and clinical levels (Perou et al., 2000; Sørlie et al., 2001), which makes it difficult to cure and assess risk factors of metastasis.

Over the last decade, there has been an increasing in our understanding of the molecular mechanisms involved in breast cancer progression, as well as the crucial role of identifying genetic alterations for the early diagnosis and treatment of breast cancer. It is well known that among all populations, an estimated 5-10% of breast cancer cases arise in individuals with mutations in genes such as *BRCA1* and *BRCA2* (Claus et al., 1991; Easton et al., 1995; Schubert et al., 1997). Furthermore, recent advances in DNA microarray technology and other methods for large-scale gene expression analysis have been adopted for both biological characterization and therapeutic planning in breast cancer (Olopade et al., 2008). Understanding the molecular biology and gene expression signatures of breast cancer are critical for developing novel approaches toward prevention and therapy.

We conducted a bioinformatic analysis to investigate the pathological mechanism in primary breast cancer and to identify corresponding signaling pathways. Furthermore, through survival analysis, we identified biological pathways that were correlated to the survival time of breast cancer patients.

MATERIAL AND METHODS

Gene expression profiles of breast cancer

The transcription profile of GSE2034 (Wang et al., 2005) was obtained from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. This data set contains 180 samples from lymph node-negative primary breast cancer patients without recurrence and 106 samples from lymph node-negative primary breast cancer patients with metastasis. In addition, among the clinical data for these samples, survival time of each patient, defined as the time to relapse or to last follow-up, were collected. Based on the GPL96 [(HG-U133A) Affymetrix Human Genome U133A Array] dataset, RNA expression of primary breast cancer cells was analyzed. The clinical protocol was approved by the National Cancer Institute of China Institutional Review Board committee, and informed consent was obtained from all patients.

Analysis of differentially expressed genes (DEGs)

We compared the expression of genes in the lymph node-negative primary breast cancer patients without recurrence and with metastasis samples. Using the spatial analysis method (SAM), only DEGs with q-values less than 0.1 were selected.

Pathway analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto, 2000) is a collection of manually drawn pathway maps of molecular interactions and reaction networks. Using SubpathwayMiner (Li et al., 2009), DEGs between the 2 sample types were enriched through the KEGG database in order to identify critical pathways involved in tumor metastasis. Pathways with false discovery rate (FDR) < 0.05 were considered to be statistically significant.

Survival analysis

Pathways that we obtained were used to as biomarkers for survival analysis. The 286 breast cancers were clustered into 2 categories: class 1 and class 2. Next, based on the survival time of these samples, survival curves for class 1 and class 2 were drawn. The log-rank test was used to compare the 2 survival curves and corresponding P values were determined.

RESULTS

DEGs identification

Based on GSE2034 data and the SAM approach, a total of 844 DEGs were identified in the lymph node-negative samples with metastasis comparing to without recurrence samples.

Signaling pathways correlated with breast cancer metastasis

Identified DEGs were subjected to KEGG pathway enrichment analysis, and were found to be enriched in various pathways. According to the P values and FDR values, the 10 most significant pathways (*i.e.*, FDR < 0.05) were collected (Table 1). Among the pathways, the extracellular matrix (ECM)-receptor interaction (P = 3.16E-05, FDR = 0.004953) and cell cycle (P = 5.59E-05, FDR = 0.004953) pathways were the most significant. Moreover, DEGs were involved in oxidative phosphorylation, DNA replication, and proteasome pathways. In addition, we found that DEGs were also enriched in several signaling pathways related to Alzheimer's disease, Parkinson's disease, and Huntington's disease.

Table 1. Results of KEGG pathway enrichment analysis of 844 differentially expressed genes.

Pathway ID	Pathway name	P value	FDR
path: 00190	Oxidative phosphorylation	0.000301	0.028406
path: 03022	Basal transcription factors	0.000522	0.048059
path: 03030	DNA replication	7.74E-05	0.028406
path: 03040	Spliceosome	0.002096	0.089964
path: 03050	Proteasome	0.0004	0.028406
path: 04110	Cell cycle	5.59E-05	0.004953
path: 04512	ECM-receptor interaction	3.16E-05	0.004953
path: 05010	Alzheimer's disease	0.001273	0.089964
path: 05012	Parkinson's disease	0.000812	0.048059
path: 05016	Huntington's disease	0.001207	0.048059

Signaling pathways correlated with the survival time of breast cancer patients

After identifying the biological pathways related to breast cancer recurrence, we further analyzed the pathways correlated with survival time. Based on the expression levels of genes involved in these pathways, we distinguished samples with long survival time from those with short survival time. Therefore, all genes in the 10 obtained significant pathways were considered as biomarkers and were adopted for survival analysis (Figures 1-5). P values of 5 signaling pathways were less than 0.1, including basal transcription factors (survival P = 0.00209), cell cycle, ECM-receptor interaction, spliceosome, and DNA replication (Table 2) pathways. Pathways with lower P values indicate that those are more closely related to the survival time of breast cancer patients.

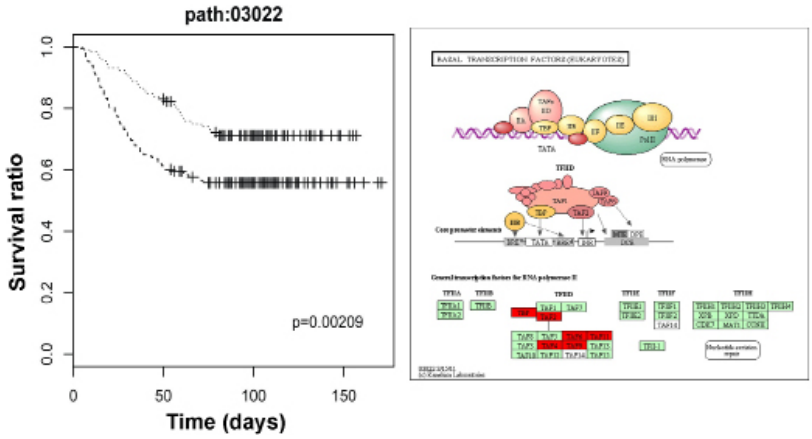

Figure 1. Survival analysis of path 03022. Left figure represents the survival curve; Right figure represents the differentially expressed genes involved in this pathway (red).

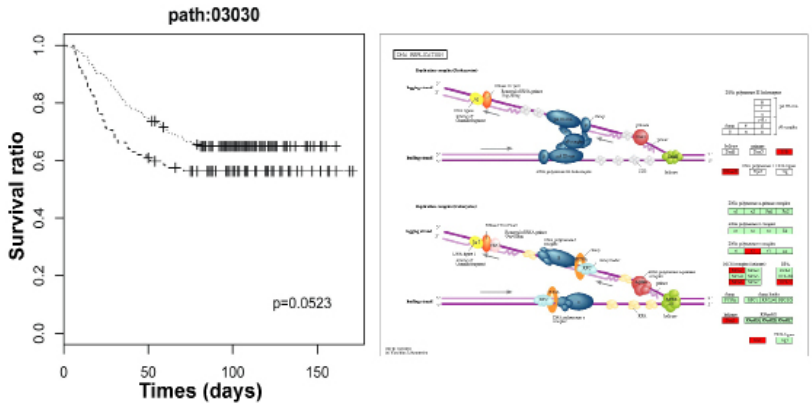

Figure 2. Survival analysis of path 03030. Left figure represents the survival curve; Right figure represents the differentially expressed genes involved in this pathway (red).

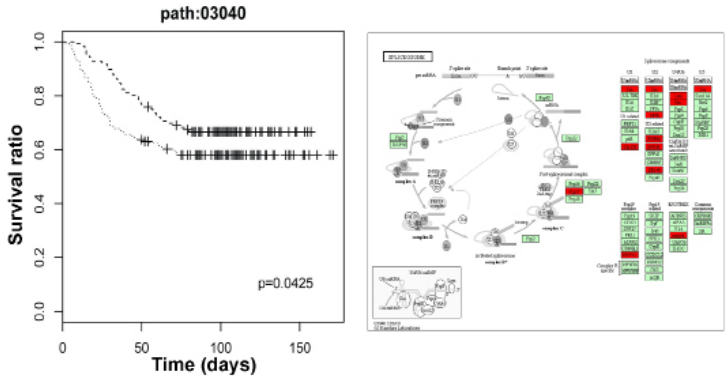

Figure 3. Survival analysis of path 03040. Left figure represents the survival curve; Right figure represents the differentially expressed genes involved in this pathway (red).

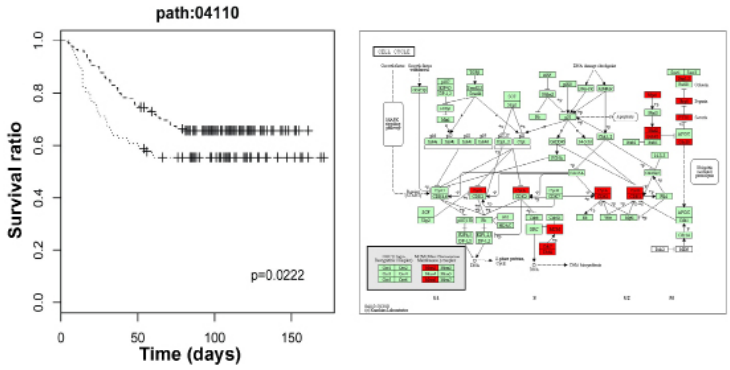

Figure 4. Survival analysis of path 04110. Left figure represents the survival curve; Right figure represents the differentially expressed genes involved in this pathway (red).

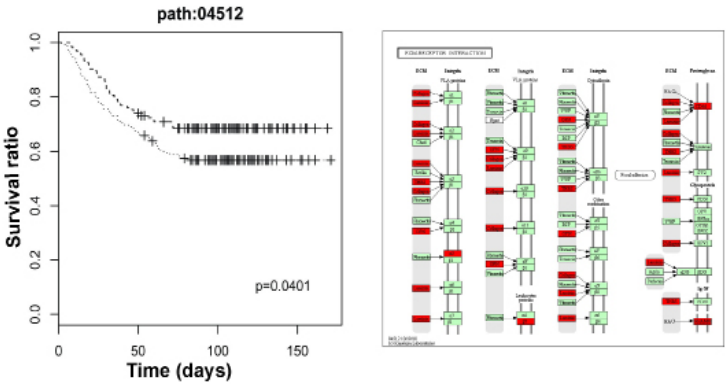

Figure 5. Survival analysis of path 04512. Left figure represents the survival curve; Right figure represents the differentially expressed genes involved in this pathway (red).

Table 2. Results of survival analysis of pathways related with survival time of breast cancer patients.

Pathway ID	Pathway name	Survival P
path: 00190	Oxidative phosphorylation	0.254
path: 03022	**Basal transcription factors**	**0.00209**
path: 03030	**DNA replication**	**0.0523**
path: 03040	**Spliceosome**	**0.0425**
path: 03050	Proteasome	0.865
path: 04110	**Cell cycle**	**0.0222**
path: 04512	**ECM-receptor interaction**	**0.0401**
path: 05010	Alzheimer's disease	0.468
path: 05012	Parkinson's disease	0.848
path: 05016	Huntington's disease	0.939

The pathways in bold mean that P value is less than 0.1.

DISCUSSION

A total of 844 DEGs in breast cancer metastasis were acquired and found to be enriched in several pathways such as the oxidative phosphorylation, DNA replication, proteasome, and ECM-receptor interaction pathways. Five pathways basal transcription factors, DNA replication, spliceosome, cell cycle and ECM-receptor interaction were related with the survival time of breast cancer recurrence.

Oxidative stress induces the secretion of matrix metalloproteinase-1, promoting the vessel growth within the tumor microenvironment (Duffy et al., 2000; Brown et al., 2000) which could increase the risk of blood-borne metastasis. p38 mitogen-associated protein kinase (MAPK) is activated by oxidative stress (Wang et al., 1998), and the phosphorylation of heat shock protein-27 by p38 MAPK has been shown to induce changes in actin dynamics (Huot et al., 1997). The proteasome, a multi-catalytic and multi-subunit protease complex, is responsible for the ubiquitin-dependent turnover of cellular proteins (Dalton, 2004; Ciechanover, 2005). Inhibition of the proteasome results in abnormal accumulation of several intracellular proteins, thereby disrupting cellular homeostasis (Codony-Servat et al., 2006) and resulting in the induction of tumor cell apoptosis (Lopes et al., 1997; An et al., 1998). The ECM modulates breast tissue homeostasis *in vivo* and regulates the growth, differentiation, and apoptosis of normal murine and human mammary epithelial cells in culture (Barcellos-Hoff et al., 1989; Petersen et al., 1992). In mammary tumors, cell-ECM interactions are disrupted whatever *in vivo* or in culture (Petersen et al., 1992; Bernfield et al., 1993).

Furthermore, among the 10 pathways correlated with breast cancer recurrence, 5 were found to be closely related to patient survival time according to survival analysis. Among them, basal transcription factors, a class of protein transcription factors that bind to specific sites on DNA to activate transcription and the constituents of basic transcriptional apparatus (Roeder, 1996; Dvir et al., 2001), are involved in the process of gene regulation, and sustaining life. DNA replication is the process of DNA copy for inheritance. A spliceosome, composed of small nuclear RNAs (snRNAs) and protein subunits, remove introns from a transcribed pre-mRNA (hnRNA) segment. A recent study demonstrated that overexpression of a splicing factor could trigger malignant transformation (Karni et al., 2007).

The cell cycle, or cell-division cycle, is the series of events that take place in a cell leading to its division and duplication. In this pathway, CDK1, a significant DEG, is considered to play an important role in cell proliferation and is expected to be associated with tumor

aggressiveness and poor prognosis (Lee and Yang, 2003; Moroy and Geisen, 2004; Sutherland and Musgrove, 2004). Some recent studies have shown that CDK1 may be required for apoptosis and regulates the cell cycle (Castedo et al., 2002; Golsteyn, 2005).

ECM receptor interaction signaling pathways have been reported to be closely related with breast cancer metastasis. Laminin plays an important role in regulating cell migration and in facilitating tumor invasion (Nielsen et al., 1983). CD44 proteins participate in a large number of related molecular processes involving specific adhesions to hyaluronate, collagen and fibronectin (Lesley et al., 1993), and cell migration (Thomas et al., 1992). Type IV collagen is involved in the regulation of mammary cell proliferation, cell attachment, and migration (Kim et al., 1994).

Although the expression of some genes found to interact with DEGs was not abnormal, these genes may nonetheless transmit signals from DEGs to downstream molecules, resulting in disruptions of important functions such as the proliferation and metastasis of cancer cells.

In summary, DEGs and their corresponding signaling pathways leading to breast cancer metastasis were identified. Five of these pathways were found to be closely related to the survial time of breast cancer patients. Our results may facilitate identification of the molecular mechanisms of breast cancer and provide options for breast cancer therapy.

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