RESEARCH ARTICLE

Identification and Functional Analysis of Differentially Expressed Genes Related to Metastatic Osteosarcoma

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Abstract

Background: To explore the molecular mechanisms of metastatic osteosarcoma (OS) by using the microarray expression profiles of metastatic and non-metastatic OS samples. Materials and Methods: The gene expression profile GSE37552 was downloaded from Gene Expression Omnibus database, including 2 human metastatic OS cell line models and 2 two non-metastatic OS cell line models. The differentially expressed genes (DEGs) were identified by Multtest package in R language. In addition, functional enrichment analysis of the DEGs was performed by WebGestalt, and the protein-protein interaction (PPI) networks were constructed by Hitpredict, then the signal pathways of the genes involved in the networks were performed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) automatic annotation server (KAAS). Results: A total of 237 genes were classified as DEGs in metastatic OS. The most significant up- and down-regulated genes were A2M (alpha-2-macroglobulin) and BCAN (brevican). The DEGs were significantly related to the response to hormone stimulus, and the PPI network of A2M contained IL1B (interleukin), LRP1 (low-density lipoprotein receptor-related protein 1) and PDGF (platelet-derived growth factor). Furthermore, the MAPK signaling pathway and focal adhesion were significantly enriched. Conclusions: A2M and its interactive proteins, such as IL1B, LRP1 and PDGF may be candidate target molecules to monitor, diagnose and treat metastatic OS. The response to hormone stimulus, MAPK signaling pathway and focal adhesion may play important roles in metastatic OS.

Keywords: Metastatic osteosarcoma - differentially expressed genes - interaction network - pathway enrichment

Asian Pac J Cancer Prev, **15 (24)**, 10797-10801

Introduction

Osteosarcoma (OS), which is the primary malignant bone tumor that arises within a bone, mostly originates in the metaphyses of long bones of adolescents and young adults (Bielack et al., 2002). OS included two types: metastatic and non-metastatic OS. All patients who was diagnosed metastatic OS, only 15-20% were treated using conventional chemotherapy regimens and surgical excision of each tumor site (Kaste et al., 1999). Meanwhile, most clinical trials in OS excluded patients with metastatic disease at presentation (Mialou et al., 2005), and the survival rate for patients diagnosed with OS in the last five years remains at 60%-70% (Pezzi et al., 1990). The cure of OS is rare after surgical treatment alone due to a high rate of systemic spread (Link et al., 1986). Therefore, exploring molecular mechanisms of OS progression will facilitate to develop effective therapeutic strategies for it.

In previous studies, the clinical manifestation of cancers such as OS is based on six essential alterations in cell physiology, including self-sufficiency in growth signals, insensitivity to growth inhibitory signals, apoptosis evasion, limitless replicative potential, sustained angiogenesis and tissue invasion (Fuchsand Pritchard, 2002; Charity et al., 2006; Kansaraand Thomas, 2007; Luo et al., 2013). And there was a correlation between E-cadherin-regulated cell adhesion and anoikis evasion among human OS cells (MG-63) (Lin et al., 2014). At the same time, chromosomal abnormalities, such as amplifications of chromosomes 6p21, 8q24, and 12q14, as well as loss of heterozygosity of 10q21.1, 10 and 13, were identified as being among the most common genomic alterations in OS (Ta et al., 2009; Smida et al., 2010). Further more, the dysfunction of a variety of tumour associated genes, such as livin, had been proved to inhibit tumor cell apoptosis through multiple ways and be involved in OS pathogenesis (Li et al., 2014). Besides, transcription factor, activator protein 1 complex (AP-1) and myc, growth factors such as transforming growth factor (TGF) and insulin-like growth factor (IGF) played significant roles in OS, and cell adhesion and migration were identified in the pathogenesis of metastatic OS (Broadhead et al., 2011). Therefore, these

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evidences suggested that the occurrence and development of metastatic OS are a complex process. Progresses have achieved in understanding the pathogenesis of metastatic OS, however, the molecular mechanism underlying its progression are still unclear.

Microarray analysis has been widely used in screening the possible targets for the treatment of metastatic OS (Diao et al., 2013). With the utilization of cDNA microarrays, the transcriptome profile of two OS cell lines has been detected, and 1098 DEGs were identified including 796 functionally characterized genes (Trougakos et al., 2010). Microarray analysis was also performed to determine histological subtype specific DEGs (Kubista et al., 2011). And the regulatory network, several signal pathways and pivotal genes were obtained in OS (LuoDeng et al., 2013). Therefore, microarray analysis is a good approach to identify key molecular events and pathways involved in metastatic OS.

In our study, microarrays were utilized for identifying the DEGs between metastatic OS samples and non-metastatic OS samples by the Multtest package. The functional enrichment analysis of DEGs was investigated by WebGestalt. Additionally, the ptotein-protein interaction (PPI) networks of the most significantly expressed genes were constructed by Hitpredict and the pathway enrichment analysis was performed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) automatic annotation server (KAAS). We anticipate that our work could improve the understanding to the underlying molecular mechanisms of metastatic OS and could provide new insights for the diagnosis and treatment of metastatic OS.

Materials and Methods

Derivation of genetic data

The gene expression profile GSE37552 (Flores et al., 2012) was downloaded from the public functional genomics database Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). Total 4 specimens, including two human metastatic OS cell line models and two non-metastatic OS cell line models were available based on the GPL570 [HG-U133_Plus_2] Platform (Affymetrix Human Genome U133 Plus 2.0 Array).

DEGs analysis

The probe-level data were converted into expression measures, and the expression values of all probes in each sample were reduced to a single value by taking the average expression value. Then the missing parts of data were imputed (Troyanskaya et al., 2001), and the complete data were standardized (Fujita et al., 2006). Under the condition of the normal tissue as the control, we applied the multtest package in R language (v.2.13.0) (Smyth, 2005) to identify the DEGs between metastatic OS samples and non-metastatic OS samples. Only the genes, with P-value<0.05 and |log fold change (FC)|>1, were screened out as DEGs.

Functional enrichment analysis

WebGestalt (Zhang et al., 2005; Duncan et al., 2010), which is a Web-Based Gene Set Analysis Toolkit, was utilized for enriching the functions of the DEGs based on the hypergeometric distribution, with the false discovery rate (FDR) less than 0.05.

Construction and analysis of interaction network

The down- and up-regulated DEGs with maximum expression degree were screened out, and in order to depict the relationship of two genes and their possible interactional objectives, Hitpredict (Patiland Nakamura, 2005; Patil et al., 2011) database was used to obtain the PPI networks, in which the two genes involved (retained the predicted objects with the highest likelihood ratio).

Pathway enrichment analysis

According to the constructed the PPI networks, the pathway enrichment analysis of genes in the PPI networks,

Table 1. The top 10 Regulated DEGs in metastatic OS with P-value<0.05

Gene.symbol	ID	logFC	P-value
A: up-regulated DEGs			
BCAN	223633_s_at	1.86	0.0354658
ADSSL1	226325_at	1.82	0.0460912
GDAP1	226269_at	1.81	0.0060979
FAM89A	226448_at	1.78	0.0322957
AP4S1	235647_at	1.7	0.0037084
GATA2	209710_at	1.7	0.0311712
PDE3A	236300_at	1.69	0.0325511
HOXB8	229667_s_at	1.61	0.0055136
TEX9	243198_at	1.58	0.0432753
FNTB	1568865_at	1.57	0.0210377
B: Down-regulated DEGs			
A2M	217757_at	-5.3	0.0200129
LOC100128252	244741_s_at	-4.87	0.0000372
TMTC1	226322_at	-4.23	0.0241149
ZSCAN18	218312_s_at	-3.89	0.0037479
TAGLN	1555724_s_at	-3.89	
0.0193384			
MOXD1	209708_at	-3.89	0.0122409
THY1	208850_s_at	-3.85	0.0478848
PRUNE2	212805_at	-3.78	0.002938
CFI	1555564_a_at	-3.75	0.0216255
ZNF667	236635_at	-3.54	0.0067286

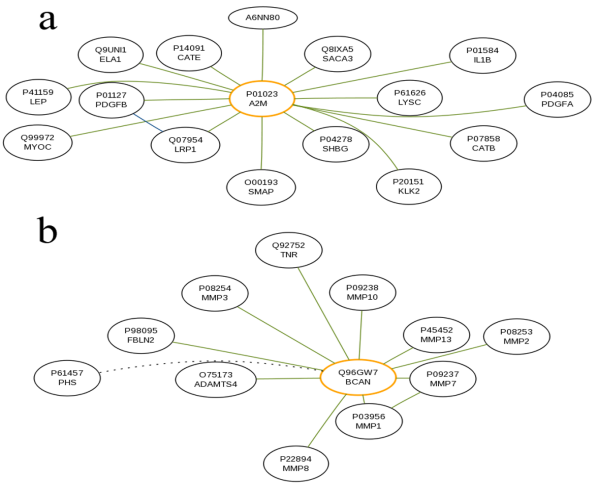

Figure 1. The PPI Networks of A2M and BCAN. A) The PPI network of A2M and its interactive proteins; **B)** The PPI network of BCAN and its interactive proteins

Table 2. Enrichment Analysis of the DEGs in Metastatic OS (FDR<0.05)

Term	Description	FDR
GO:0009725	response to hormone stimulus	2.36E-05
GO:0010544	negative regulation of platelet activation	3.97E-05
GO:0009719	response to endogenous stimulus	5.54E-05
GO:0048545	response to steroid hormone stimulus	8.58E-05
GO:0010543	regulation of platelet activation	5.53E-04
GO:0010033	response to organic substance	6.68E-04
GO:0032101	regulation of response to external stimulus	7.16E-04
GO:0050865	regulation of cell activation	0.001259
GO:0048585	negative regulation of response to stimulus	0.001829
GO:0044057	regulation of system process	0.002164
GO:0019216	regulation of lipid metabolic process	0.003208
GO:0050866	negative regulation of cell activation	0.008183
GO:0030195	negative regulation of blood coagulation	0.017146
GO:0051384	response to glucocorticoid stimulus	0.021877
GO:0050819	negative regulation of coagulation	0.025074
GO:0031960	response to corticosteroid stimulus	0.03077
GO:0007264	small GTPase mediated signal transduction	0.031
GO:0045833	negative regulation of lipid metabolic process	0.031512

*FDR is the abbreviation of false discovery rate

Table 3. The Signal Pathways in the Interaction Network of A2M

Term	*P*-value	Genes
hsa04010:MAPK signaling pathway	1.50E-04	CDC42, PDGFB, GRB2, PDGFA, IL1B, RAP1B, NGF
hsa04060:Cytokine-cytokine receptor interaction	0.001296	LEP, IL4, PDGFB, PDGFA, IL1B, IL10
hsa05200:Pathways in cancer	0.003513	CDC42, PDGFB, GRB2, PDGFA, KLK3, MMP2
hsa04510:Focal adhesion	0.003759	CDC42, PDGFB, GRB2, PDGFA, RAP1B
hsa04660:T cell receptor signaling pathway	0.005101	IL4, CDC42, GRB2, IL10
hsa04722:Neurotrophin signaling pathway	0.007495	CDC42, GRB2, RAP1B, NGF
hsa04630:Jak-STAT signaling pathway	0.013797	LEP, IL4, GRB2, IL10
hsa05214:Glioma	0.018229	PDGFB, GRB2, PDGFA
hsa04540:Gap junction	0.034741	PDGFB, GRB2, PDGFA
hsa04912:GnRH signaling pathway	0.041433	CDC42, GRB2, MMP2
hsa04670:Leukocyte transendothelial migration	0.057878	CDC42, RAP1B, MMP2
hsa05310:Asthma	0.092792	IL4, IL10

where the up- and down-regulated DEGs with maximal expression levels located, was performed by using KAAS (Ye et al., 2006).

Results

Screening DEGs in metastatic OS

Basing on the microarray analysis, a total of 237 genes were detected to be differentially expressed in metastatic OS samples, including 94 up-regulated genes and 143 down-regulated genes. In the DEGs, A2M (Alpha-2-Macroglobulin) was most significantly expressed in up-regulated genes, and BCAN (brevican) was the most significant down-regulated genes. The top 10 up-regulated DEGs and top 10 down-regulated DEGs were listed in Table 1.

Functional enrichment analysis of the DEGs

To analyze the function of the DEGs in metastatic OS, the DEGs were mapped to the WebGestalt. Table 2 displayed 18 functions of the DEGs, and the most significant function is response to hormone stimulus.

Network constructing

A2M and BCAN were screened out as the most significant genes in the DEGs, and Hitpredict was used to construct the PPI networks of A2M and BCAN. The network of A2M included 15 genes, such as IL1B (Interleukin), LRP1 (low-density lipoprotein receptor-related protein 1) and PDGF (platelet-derived growth factor), while there were 11 proteins in the network of BCAN (Figure1), such as matrix metalloproteinases (MMP2) and Fibulin2 (FBLN2).

Pathway enrichment analysis

In order to gain further insights into the changes of biological pathways in metastatic OS, the proteins of the network were analyzed by KAAS software. Consequently, only 12 significant pathways of the proteins involved in the network of A2M were obtained (Table 3), and MAPK (Mitogen-activated protein kinase) signal pathway was the most significant pathway.

Discussion

Due to the low cure rates and lacking of the specific drugs with no toxicity for metastatic OS, exploring the mechanism and the effective prevention strategy of OS is urgent for us. In this study, we analyzed the DEGs between metastatic OS samples and non-metastatic OS samples. Finally, 237 genes were screened out as the DEGs. Based on the DEGs we obtained, the function analysis showed

that DEGs were significantly related to the function of the response to hormone stimulus.

In previous studies, the regulation of hormone was supposed to be an vital function in the progression of cancers, such as prostate cancer (Linja et al., 2001). The etiology of breast cancer is becoming clearer by investigating the molecular alterations in germ line and somatic cell genes, and the interaction of these genes with steroid hormones (MacMahon et al., 1973; Hulkaand Moorman, 2008). Moreover, bisphenol A (BPA) is an environmental estrogen and its exposure may interact with the -22 G/C polymorphism of the LOX gene, thereby increasing the risk of OS (Jia et al., 2013). Therefore, these evidences suggest that the response to hormone stimulus may play a vital role in metastatic OS, but the mechanism needs to further study.

Furthermore, we found that A2M was the most significantly expressed in up-regulated genes, and it was also identified in the previous research of OS (LuoDeng et al., 2013). In general, A2M is a high-molecular weight homotetrameric glycoprotein and functions as a physiological guardian (Rehman et al., 2013). Many researches focus on its function in the Alzheimer's disease and depression (Blennow et al., 2000; Fujita et al., 2003), and few are in cancer even OS. Consequently, in order to articulate the roles of A2M in metastatic OS, we performed PPI network and identified its interactive proteins, for instance, IL1B, LRP1 and PDGF. IL1B encodes the proinflammatory cytokine IL-1β with multiple biological effects (Lee et al., 2003), and represents the potential effects in the gastric cancer (Kulmambetova et al., 2014). Very recent study also confirms that the genetic polymorphisms of IL1B are strongly associated with OS risk (He et al., 2014). LRP1 is a ubiquitously expressed endocytic receptor belonging to the LDL-receptor family (Herzand Strickland, 2001). LRP-1 can promote cancer cell invasion via supporting ERK and inhibiting JNK signaling pathways (Langlois et al., 2010), and has been identified as a molecular signaling partner for platelet-derived growth factor receptor (PDGFR) (Boucherand Gotthardt, 2004). Moreover, PDGFR is considered as a therapeutic target and a prognostic marker for imatinib mesylate therapy in OS (Kubo et al., 2008). PDGF released from platelets plays an important role in promoting OS cell growth by activating the PDGFR-Akt signaling axis (Takagi et al., 2014). Hence, these genes may process important functions in metastatic OS.

In addition to the interactive regulation of the genes, several significantly pathways correlated with OS were also found. MAPK signal pathway, the most significant one, plays a crucial role in cancer progression including angiogenesis, proliferation, apoptosis and metastasis (Tingting et al., 2010). It also may be involved in OS by activating cyclin D1 (Hu et al., 2001), and its activation was closed related to the therapeutic strategy in OS (Yang et al., 2008). Additionally, the pharmacological inhibition of the MAPK pathway could enhance the antitumoral effect of mammalian target of rapamycin (mTORC1) inhibition by rapamycin in cancer cells (Carracedo et al., 2008). Inhibition of mTORC1 and mTORC2 by the combination of sorafenib and everolimus contribute to anti-tumor activity in OS preclinical models (Pignochino et al., 2013). Meanwhile, focal adhesion and pathways in cancer were included in these pathways. Taking focal adhesion as example, focal adhesion kinase signaling plays a pivotal role in anti-tumor effects of Yangzheng Xiaoji in human OS (Jiang et al., 2013), and inhibition of focal adhesion kinase induces apoptosis in OS SAOS-2 cells (Wang et al., 2014). Therefore, these evidences suggest that MAPK signal pathway and focal adhesion are more likely to be the crucial mechanisms involved in metastatic OS.

In consequence, our studies analyze the DEGs in metastatic OS tissues compared to non-metastatic OS controls and identify the functions of the DEGs by a computational bioinformatics approach. Response to hormone stimulus may process an important function in metastatic OS. Meanwhile, A2M and its interactive proteins, such as IL1B, LRP1 and PDGF may play a vital role in metastatic OS and be considered as potential targets for the treatment of it. Besides, MAPK signal pathway, focal adhesion and other pathways enriched by proteins in the network may be crucial mechanisms involved in metastatic OS. Our research may provide a new strategy in the medical therapy of metastatic OS. However, no experimental validations and less sample size are limitations in the present study, and further experiments are still necessary for confirming our conclusion.

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