Short Communication

Screening for Differentially Expressed Genes of Gastric Stromal Tumor Originating from Muscularis Propria

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Introduction

Gastric stromal tumor (GST) is a set of gastrointestinal mesenchymal tumors those originate from interstitial cells of Cajal. Its early diagnosis and treatment are critical to prognosis.[1] The occurrence of GST remains obscure; this study used Affymetrix expression spectrum chip to detect the gene expression spectrum of GST and explore new molecular target that is used in the treatment and prognosis of GST.

Methods

Selection of clinical samples

Six patients with GST (two males and four females, aged 41–71 years, with average age of 60 years) were enrolled in this study. The size of GST ranged from 2.5 to 5.0 cm, with average size of 3.8 cm. Four cases had mitotic index <5/50 high power field (HPF) and two cases had mitotic index <10/50 HPF. Tissue samples of GST that were treated with endoscope and laparoscope as well as its surrounding tissues were collected, three samples for each group. The tumor tissue and corresponding surrounding tissue in each sample were extracted. The Research Ethics Committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University approved this study (No. [2016]172). Informed consents were obtained from all the enrolled patients.

Test materials and reagent

Equipment and reagent of Eukaryotic Poly-A RNA Control Kit (Affymetrix Company, USA); MessageAmp™ Premier RNA Amplification Kit (Ambion Company, USA); Eukaryotic Hybridization Control Kit (Affymetrix Company, USA); Hybridization, Wash, and Stain Kit (Affymetrix Company, USA); PCR Biometra (MJ Company, USA); and Gene Chip® Scanner 3000 (Affymetrix Company, USA) were used.

Total RNA extraction and probe preparation

This test was performed in CapitalBio Technology Co., Ltd. (Tsinghua University, China). Trizol total RNA extraction kit was used to extract and purify total RNA. We started from the total RNA of GST and surrounding tissues and synthesized the first chain of complementary DNA (cDNA) through reverse transcription using T7 Oligo (dT) primer that contains T7 promoter sequence as primer. Then, the first chain of cDNA was used as template to synthesize the second chain of cDNA. The second chain of cDNA was used as template to use T7 enzyme mix to synthesize cRNA through *in vitro* transcription and by adding biotin labeling. cRNA was purified with magnetic bead and cRNA was quantified and segmented into size suitable for hybridization.

Chip hybridization, clean-up, and staining

Segmented cRNA was prepared into hybridization reaction system to initially prehybridize the chip for 10 min. Then, the chip was put into the hybridization oven in a balanced manner and was hybridized for 16 h cyclically with hybridization solution that is from Affymetrix Human Genome U133 Plus 2.0 Array expression profile

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chip (Affymetrix Company, USA). After this, the chip was injected cleaning and staining fluids for cleaning and staining.

Scanning, data extraction, and bio-information analysis of chip

The fluorescence ship scanner (Gene Chip® Scanner 3000) from Affymetrix Company was used to scan chip. Data preprocessing was done with robust multi-array average algorithm, and the data were performed to analyze differentially expressed gene using the R pack of significance analysis of microarray. According to criteria for selecting differential gene ($q \leq 5\%$ and fold change ≥ 2 or ≤ 0.5), it was judged as differentially expressed gene of GST and surrounding tissues. Finally, PANTHER/KEGG (Protein Analysis Through Evolutionary Relationships/Kyoto Encyclopedia of Genes and Genomes, Kyoto University, Japan) database and Gene Ontology (GO) analysis software were used to analyze these differentially expressed genes bioinformatically to find the molecular functions and biological pathways involved by these differentially expressed genes.

Results

Total RNA extraction analysis

The six pairs of tissue samples were detected with spectrophotometer; their A260/A280 ratios were at the range from 1.8 to 2.0. Agarose gel electrophoresis found two clear 28s/18s ribosomal RNA bands, indicating that the total RNA purity and integrity were high and met experimental requirements.

Analysis of differentially expressed gene

According to differential gene selection criteria: $q \leq 5\%$ and fold change ≥ 2 or ≤ 0.5, the six pairs of samples totally had 3293 common differentially expressed genes, including 2588 genes upregulated and 705 downregulated. Part of the differentially expressed genes is shown in Table 1.

Pathway and Gene Ontology analysis for common differentially expressed genes of gastric stromal tumor and its surrounding tissue

By analyzing common differentially expressed genes with PANTHER software, it was found that two groups of differentially express genes and three pathways were involved, including cadherin signal pathway, Wnt signal pathway, and angiogenesis.[2]

By searching KEGG database, it was found that 13 pathways were involved, including carbon metabolism and cancers, Rap1 signal pathway, extracellular matrix receptor interaction, phosphatidylinositol signal system, Ras signal pathway, phosphatidylinositol 3-kinase (P13K)-AKT signal pathway, cell adhesion molecules, and several pathways closely associated with development of tumors.[3]

By searching and analyzing the biological process, cell composition, and molecular function of differentially expressed genes using GO function analysis software, it was found that a significant number of differentially expressed genes had relationship with extracellular matrix and basement membrane in the aspect of cell composition. Most differentially expressed genes were combined with calcium ion, growth factor, and metal and were related to transmembrane receptor protein kinase's activity, cell adhesion molecules, and nucleic acid kinase in the aspect of molecular function. From the perspective of biological process, many genes had participated in the various biological processes associated with tumor, such as cell differentiation, cell or intercellular adhesion, calcium ion transmembrane activity regulation, cell proliferation regulation, cell migration regulation, metabolism process of guanosine triphosphate and diphosphate nucleic acid, and associated signal transmission, and electrically coupled cell communication.

Discussion

In this study, Affymetrix messenger RNA expression spectrum chip was used to compare the gene expression spectrum difference between GST tissue and its corresponding surrounding tissue, and the results showed 3293 common differentially expressed genes between the two groups, including 2588 upregulated and 705 downregulated. Among the upregulated differentially expressed genes, the upregulation of *DPP10* was most significant, with fold change value up to 244.4. *DPP10* gene is a member of the serine endopeptidase family. *DPP10* can gate-control potassium ion pathway by regulating voltage to vary conformation, affecting cell proliferation and differentiation, cell cycle progression, and cell apoptosis. A previous study showed that *DPP10* gene is differently expressed in various tissues and organs and is related to the genesis of a variety of diseases and tumors;[4] we presume that *DPP10* that was obviously upregulated in this study might participate in the genesis and development of GST through certain mechanism, for which the details are pending for further studies.

Both *KIT* and *PDGFRA* genes were found as upregulated expressions in this study. As the gain-of-function mutation of *KIT* and *PDGFRA* has been proved in study, they can be treated as the two leading genesis mechanisms for most GSTs for the relation of their overexpression or mutation to the genesis and development of GST. Furthermore, imatinib, as a tyrosine kinase inhibitor for c-KIT or PDGFRA, is extensively used in treating GST.[5] We also found in this study that the expression of *ETV1* gene, a member of the ETS family, is obviously upregulated. Each ETS family member has a highly conservative DNA-binding domain (ETS domain) that regulates gene transcription, adjusts cell proliferation and differentiation, and participates in the genesis and development of a variety of tumors. In our study, we found that the over expression of *DKK4* might promote the proliferation of tumor cells with unclear mechanism and that there is over expression in genes related to cell adhesion, such as *FAT3*, *PCDH* family (including *PCDHAI*, *PCDHB16*, and *PCDH14*), *CDH11*, and *DCHS1*, and they

Table 1: Top ten genes with significant differential expression of GST and its surrounding tissues

Number	Fold change values	Gene symbol	Gene title
Upregulated genes			
1	244.3996	*DPP10*	Dipeptidyl-peptidase 10 (nonfunctional)
2	168.8299	*F2RL2*	Coagulation factor II (thrombin) receptor-like 2
3	83.3715	*FAM19A1*	Family with sequence similarity 19 (chemokine (C-C motif)-like), member A1
4	78.7709	*DKK4*	Dickkopf Wnt signaling pathway inhibitor 4
5	71.1347	*ETV1*	ETS variant 1
6	69.5332	*NRK*	Nik-related kinase
7	47.4055	*LY6H*	Lymphocyte antigen 6 complex, locus H
8	47.185	*PRKCQ*	Protein kinase C, theta
9	45.3573	*NPFFR2*	Neuropeptide FF receptor 2
10	41.2274	*ANO1*	Anoctamin 1, calcium activated chloride channel
Down-regulated genes			
1	0.0038	*ADH1B*	Alcohol dehydrogenase 1B (Class I), beta polypeptide
2	0.006	*ALDH1A1*	Aldehyde dehydrogenase 1 family, member A1
3	0.0114	*MT1M*	Metallothionein 1M
4	0.0131	*SEMA3C*	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C
5	0.0134	*CXCL14*	Chemokine (C-X-C motif) ligand 14
6	0.0158	*SFRP2*	Secreted frizzled-related protein 2
7	0.0184	*ADH1C*	Alcohol dehydrogenase 1C (Class I), gamma polypeptide
8	0.0225	*EPB41L4B*	Erythrocyte membrane protein band 4.1 like 4B
9	0.057	*SDC4*	Syndecan 4
10	0.0582	*GPR56*	G protein-coupled receptor 56

GST: Gastric stromal tumor.

participate in the adhesion, migration, differentiation, and signal transmission of cells by adjusting the intercellular interaction and interaction between cell and extracellular matrix; they might also be relevant to the progression of GST. In addition, our test also detected clear upregulation of genes related to cell growth and differentiation, such as *JAK3*, *BAX*, *FGFR1*, and *ETV5*, which can promote abnormal proliferation and differentiation of cells. Their over expression might be a promoting factor for the development of GST. This study found that downregulated gene expression was mainly reflected in functions such as tumor inhibition, intercellular signal transmission, stress, and immune defense.

In addition, by searching PANTHER database, it can be found that these differentially expressed genes are involving a number of signal transmission pathways related to the genesis and development of tumors, such as Wnt signal pathway and angiogenesis, which is correlated with tumor angiogenesis. ETV played such a role.[6] Furthermore, the result of KEGG pathway analysis showed that genes in GST differentially expressed are involved in pathways related to carbon metabolism, amino acid biosynthesis, and cell adhesion have differential gene expression, indicating that involved substance metabolism and intercellular adhesion can promote cell growth and eventually tumor genesis; for those pathways that are closely related to tumor genesis, such as Rap1 signal pathway, sulfur ester acyl inositol signal system, Ras signal pathway, and P13K-ART signal pathway, the involved genes are also expressed differentially, suggesting that the genesis of GST has activated a number of tumor-related pathways that regulate the genesis and development of GST. However, exact tumorigenesis mechanisms and their interactions are still required in further studies and verification.

This study showed that there were significant differentially expressed genes in GST and its surrounding tissues. If further studies can be performed to explore these differentially expressed genes in GST and their exact tumorigenesis mechanism and the interactions to find new molecular biomarkers for GST diagnosis and prognosis, new insights of GST diagnosis and treatment can be made.

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Conflicts of interest

There are no conflicts of interest.

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