

Gastric cardia adenocarcinoma pathway analysis

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ABSTRACT

Gastric cardia adenocarcinoma (GCA) is one of the few malignancies with unexplained reasons, which have increased abruptly in developed countries. The purpose of this study was to determine the pathways in GCA to identify a new biomarker. Therefore, by comparing gene expression in GCA group with normal control identified important pathways. Gene expression data were extracted from the beforehand investigations then differentially expressed genes utilized in the DAVID program to explore and find related pathways. Our findings contain 367 gene names. Out of these 367 proteins, 199 were found to be exclusively expressed in GCA; whereas 168 proteins were detected down-regulated or silenced. The GCA associated diseases are based on the differently expressed genes made up of disease pathways related to colorectal cancer, small cell lung cancer, breast cancer and H. pylori infection stomach cancer. KEGG pathways related to GCA contained cell cycle, p53 signaling pathway, DNA replication, toll-like receptor signaling pathway and some other diseases. The GO-discovered categories also demonstrated most biological process and molecular function related to cancer. Up until now, there is no report to introduce influential biomarkers in GCA. Therefore, the deregulated genes identified in GCA patterns might be helpful for diagnosis, prognosis and therapies for gastric cancer but validation of these biomarkers is necessary.

Keywords: Gastric cardia adenocarcinoma, Pathway, Biomarker.

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Introduction

Gastric cancer is the second most common cause of cancer death worldwide (1). Because most patients are diagnosed at an advanced stage, where treatment options are limited, the 5-year survival rate is below 20%. Indeed, precancerous lesions are often difficult to differentiate from gastric carcinomas in biopsy samples by conventional histopathologic analysis. In fact, experienced pathologists often

disagree in distinguishing invasive carcinoma from high-grade dysplasia in gastroscopic biopsy specimens. Currently, available serological tumor markers such as carcino embryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9) (2-5), for the early detection of gastric cancer is not enough sensitive and specific. Therefore, new early stage detection techniques, treatment options and knowledge about the molecular mechanisms of gastric cancer are needed. In this study, we focused on gene expression to improve the understanding, diagnosis, and follow-up of the progression of GCA.

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Gastric cardia adenocarcinoma

More than 90% of malignant tumors in the stomach are adenocarcinomas and less frequent tumors of the stomach include lymphomas, carcinoids and gastrointestinal stromal tumors respectively (1). Gastric adenocarcinomas are biologically and genetically very heterogeneous (6) that tumors arising in the stomach may have distinct etiologies in which gastric cardia adenocarcinoma (GCA) show biologic, epidemiologic, and clinic pathologic features more closely resembling esophageal adenocarcinomas (EACs) than gastric noncardia adenocarcinoma (GNCA). The cardia anatomically lies between the end of the esophagus and the body of the stomach. Moreover, genetically reported the P53 mutation spectrum in GCA more closely resembled EAC than GNCA (7). A number of other genetic alterations have been reported in gastric cancer, including CDH1 (8), β -catenin (6), TFF1 (9), and Met (10). Further, canonical oncogenic pathways such as E2F, K-RAS, p53, and Wnt/ β -catenin signaling are also known to be deregulated with varying frequencies in gastric cancer (11, 12).

Epidemiology and risk factors

GCA is one of the few malignancies that has increased abruptly in developed countries in recent years for reasons that are yet unexplained (13, 14). The wide variation in incidence across different geographical areas and higher proportion of GCA are two main characteristics of gastric cancer in Iran. It is the most common cancer in north and northwest of Iran. A high prevalence of *H.pylori* infection, high dietary intake of salt and smoking are the main environmental factors of gastric cancer in Iran. Gastroesophageal reflux disease, red meat and dairy products are other contributing factors in populations with a higher incidence of GCA (15). Increased age, male gender, a family history of upper gastrointestinal tract cancer, thermal damage from hot food (16) have all been consistent risk factors for

GCA (16-24). A recent genome-wide association study of germline DNA found a common gene (PLCE1) associated with risk for both GCA and ESCC (25). Wang et al. identified genomic differences between gastric cancer by anatomic subtypes to development of appropriate targeted strategies for early detection, prognosis, and therapy (26). Therefore, the aim of this study was to investigate GCA at the molecular level using pathway analysis. Consequently, this makes it relatively easy to find candidate marker proteins for GCA. Beside study based systems provide convenient biomarkers of diagnosing cancer as well as drug targets. In addition, tumor therapy requires information about molecular alterations. Thus, we studied different gene expression of GCA in literature.

Gene expression data from GCA

During the last decade, there has been an exponential increase in the number of studies analyzing cancer tissue. In this study, data were extracted from Wang et al. investigation (26). All genes were significantly altered (up-regulate, down-regulate, silent and new expression gene) between gastric cardia adenocarcinoma tissues compared to normal tissues. For GCA, a total of 367 genes were differentially expressed between tumors and their matched normal samples. Of these genes, 199 genes were up-regulated and 168 were down-regulated. So according to these data, some analyses to find out early diagnosis or drug targets were performed.

In order to carry out a retrospective meta-analysis, the functional annotations using UniProt accession numbers (<http://www.uniprot.org>), which is a publicly available web-based tool, to search for annotations that are significantly associated to the list of GCA related proteins was used.

We also used DAVID bioinformatics resources 6.7 (the Database for Annotation, Visualization, and Integrated Discovery) (27), "<http://david.abcc.ncifcrf.gov/>", a comprehensive

set of functional annotation tools for understanding the biological meaning behind large list of genes, to obtain gene ontology and KEGG pathway information for differential genes between the GCA and control pattern. Differentially expressed genes that were similar and different between the GCA and control were compared. The DAVID gene classification tool functionally analyzes a large number of genes in a high-throughput fashion by classifying them into gene groups based on their annotation term co-occurrence. This classification is accomplished with p-value (EASE Score, an alternative name of fisher exact statistics in DAVID system, referring to one-tail Fisher exact probability value used for gene-enrichment analysis) for each enriched annotation terms.

Cancer cells do not invent new pathways; they use pre-existing pathways in different ways or they combine components of these pathways in a new fashion. Gene clustering based on functions illustrate correlated expression patterns (28, 29) and analyzing the gene-expression data might reveal the organizational pattern of gene expression in cancer, which might help us to identify new potential drug targets. Therefore, in this study we have been utilizing classification software for mining gastric cancer data. The diseases related to expressed genes using GENETIC_ASSOCIATION_DB_DISEASE was detected the most diseases related to the expressed genes in GCA pattern versus control made up of diseases pathway related to cancer (BIRC5, BUB1B, APOE, IL8, MMP7, PCNA, TFF2 and...), colorectal cancer (CD14, ADH1B, AURKA, CTSB, FAM46A, GSTA1, GSTA4, APOE, IGFBP3, IL8, MMP7, MMP9, MDK, PLA2G2A, PLA1, PCNA, RNASE1, SOD2, TYMS and TFRC), H. pylori infection stomach cancer (TIMP1, MMP7 and MMP9), Small cell lung cancer and breast cancer (MAD2L1, TTK, UGT2B15, ADH1B, AURKA, BUB1B, COL18A1, GSTA1, GHR, APOE, IGFBP3, IL8, LEPR, MMP9, PON2, SST, SOD2, TYMS, TFRC). Some of the differentially expressed genes reported in GCA were also

dysregulated in a similar pattern as colorectal cancer, small cell lung cancer and breast cancer suggests that despite their differences in cell types, these cancers likely share common genetic and/or environmental factors in their etiology. Such evidence for a common genetic influence is evident by results from a genome-wide association study, which found a shared susceptibility locus in PLCE1, CDC25B and COL1A2 for both GCA and ESCC (26, 30). While progression of H. pylori induced superficial gastritis to chronic atrophic gastritis, intestinal metaplasia and dysplasia is the main pathologic event in majority of GNCA, GCA show less prominent and even revers relationship with H.pylori infection and subsequent atrophic gastritis. In contrast, recent study in Ardabil supported that H. pylori infection could be a putative risk factor for GCA. Because the incidence of GCA in the Ardabil region is higher than European countries, U.S.A., Japan and Korea (31) and more than 98% of people age 40 years old and above have been infected with H. pylori and almost all of them had H. pylori associated chronic gastritis involving the antrum, corpus, and cardia, which consequently up to 35% have concomitant corpus atrophic gastritis (32).

Pathway Analysis Using DAVID

The KEGG pathway analyses of the significantly expressed genes using DAVID are shown in tables 1 and 2. The GCA pattern versus control analysis revealed that different pathways were related to the following up-regulated expressed genes (Table 1) that contained ECM-receptor interaction, cell cycle, focal adhesion, p53 signaling pathway (CCNB1, CCNB2, CCNE1, IGFBP3, RRM2, SERPINB5, SFN and THBS1), small cell lung cancer (E2F3, COL4A1, COL4A2, CCNE1, FN1 and LAMB1), DNA replication, toll-like receptor signaling pathway,

bladder cancer (E2F3, IL8, MMP9 and THBS1), oocyte meiosis, leukocyte trans endothelial migration, systemic lupus erythematosus, pathways in cancer (E2F3, BIRC5, COL4A1, COL4A2, CCNE1, FN1, IL8, LAMB1, LEF1 and MMP9), complement and coagulation cascades. Over the past few decades, many studies have identified and characterized pathways within gastric cancer that the number of them associated up-regulated genes include the cell cycle, cellular growth and proliferation, cell cycle checkpoint, extracellular matrix remodeling, and angiogenesis (eg, Wnt signaling and cell cycle checkpoint pathways, such as SULF1, SFRP4, LEF1, TOP2A, and CDC2 (33-37)), and the integrin signaling pathway (ARPC1B, COL1A1, COL4A1, FN1, and LAMB1). Some genes are also related to adaptive immune responses (eg, CD14) and tumor metastasis (eg, CD9). The most KEGG pathway according to table 2, were found in down-regulated genes contribute to pathways in metabolism of xenobiotics by cytochrome P450, drug metabolism, arginine and proline metabolism, butanoate metabolism, fatty acid metabolism, retinol metabolism, nitrogen metabolism, histidine metabolism, prion diseases, tryptophan metabolism, valine, leucine and isoleucine degradation, tyrosine metabolism and steroid hormone biosynthesis.

The common down-regulated genes previously found in GCA are consistent with other studies on gastric cancer, such as AKR1B10, ALDH3A1, ATP4B, CA2, IGFBP2, KLF4, MUC5AC, MUC6, TFF1, and TFF2 (35,38,39). Important pathways in down-regulated genes were mainly involved in metabolic pathways, digestive system development, or mucosal integrity. Several genes are thought to have specific functions in gastric epithelium, such as PGC and GIF, implying that dedifferentiation is a common feature of carcinogenesis (26, 33). BUB1B is also a

spindle-assembly checkpoint gene, which is a susceptibility gene for this gastric tumor (26).

Table 1. The KEGG pathways related to the up-regulated expressed genes in GCA pattern

KEGG Pathway	Number of genes	%	P-Value
ECM-receptor interaction	15	8.2	<0.0001
Cell cycle	14	7.7	<0.0001
Focal adhesion	16	8.8	<0.0001
p53 signaling pathway	8	4.4	<0.0001
Small cell lung cancer	6	3.3	0.011
DNA replication	4	2.2	0.02
Toll-like receptor signaling pathway	6	3.3	0.023
Bladder cancer	4	2.2	0.03
Oocyte meiosis	6	3.3	0.03
Leukocyte transendothelial migration	6	3.3	0.04
Systemic lupus erythematosus	5	2.7	0.075
Pathways in cancer	10	5.5	0.08
Complement and coagulation cascades	4	2.2	0.099

Table 2. The KEGG pathways related to the down-regulated expressed genes in GCA pattern

KEGG Pathway	Number of genes	%	P-Value
Metabolism of xenobiotics by cytochrome P450	8	0.6	<0.0001
Drug metabolism	8	0.6	<0.0001
Arginine and proline metabolism	5	0.3	0.003
Butanoate metabolism	4	0.3	0.007
Fatty acid metabolism	4	0.3	0.011
Retinol metabolism	4	0.3	0.024
Nitrogen metabolism	3	0.2	0.029
Histidine metabolism	3	0.2	0.044
Prion diseases	3	0.2	0.062
Tryptophan metabolism	3	0.2	0.078
Valine, leucine and isoleucine degradation	3	0.2	0.092
Tyrosine metabolism	3	0.2	0.092
Steroid hormone biosynthesis	3	0.2	0.099

Table 3. GO-discovered categories for the genes that up-regulated in the GCA pattern

GO categories	GO name	No. of genes	P-value
Biological process			
	cell cycle	31	<0.0001
	regulation of cell cycle	14	<0.0001
	response to stimulus	63	<0.0001
	developmental process	68	<0.0001
	epidermis development	9	0.001
	collagen metabolic process	7	<0.0001
	intracellular signaling cascade	20	0.12
	cell cycle checkpoint	8	<0.0001
	extracellular structure organization	13	<0.0001
	response to nutrient	7	0.005
	response to vitamin	5	0.007
	apoptosis	15	0.009
	regulation of biological process	96	0.016
	response to DNA damage stimulus	10	0.027
Cellular constituent			
	extracellular region	71	<0.0001
	spindle	14	<0.0001
	nuclear part	28	0.059
	membrane	72	0.9
Molecular function			
	glycosaminoglycan binding	14	<0.0001
	enzyme regulator activity	23	<0.0001
	cytokine activity	8	0.005
	G-protein-coupled receptor binding	4	0.12
	transcription regulator activity	11	0.97
	kinase activity	7	0.89

Gene ontology analysis using DAVID

The GO-discovered categories using DAVID analysis for up-regulated expressed genes (Table 3) between GCA pattern and the control pattern comparisons were predominantly grouped into functional classes of glycosaminoglycan binding, enzyme regulator activity, cytokine activity, G-protein-coupled receptor binding, transcription regulator activity and kinase activity. The most important biological process are cell cycle, regulation of cell cycle, response to stimulus,

developmental process, epidermis development, collagen metabolic process, intracellular signaling cascade and cell cycle checkpoint. Cellular constituent of down regulated expressed genes are a part of extracellular region, spindle, nuclear part and membrane. The genes that were uniquely down-regulated expressed between the GCA pattern and the control were as shown in Table 4, predominantly grouped into biological process, including oxoacid metabolic process, lipid metabolic process, response to stimulus, response to reactive oxygen species, response to nutrient levels, and response to drug. Functional classes contain mitochondrial part, oxidoreductase activity, cofactor binding, lipid binding, ion binding, DNA binding and signal transducer activity. Cellular constituent of down regulated expressed genes are a part of endocytosis, extracellular region, plasma membrane part, insoluble fraction, membrane and cytoplasm. All these up-regulated expressed genes are functionally related, or members of the same pathway or protein complex, which able us to identify genes that share similar expression patterns across a variety of experimental conditions.

For genes dysregulated significantly in GCA, we note a couple of interesting examples here. E2F3 (E2F transcription factor 3) showed up-regulated in GCA patients. E2F3, transcription factor E2F/dimerisation partner (TDP), winged helix repressor DNA-binding, play an essential role in most cancer pathway such as cell cycle, pancreatic cancer, glioma, prostate cancer, melanoma, bladder cancer, chronic myeloid leukemia, small cell lung cancer, non-small cell lung cancer (40, 41). Other genes up-regulated significantly in GCA only, but not in GNCA is MAD1L1 (MAD1 mitotic arrest deficient-like 1) play as cell cycle checkpoint present in different diseases like lymphoma, somatic, prostate cancer (42).

Table 4. GO-discovered categories for the genes that down-regulated in the GCA pattern

GO categories	GO name	Number of genes	P_Value
Biological process	oxoacid metabolic process	19	<0.0001
	lipid metabolic process	17	0.002
	response to stimulus	44	0.009
	response to reactive oxygen species	6	<0.0001
	response to nutrient levels	7	0.008
	response to drug	6	0.042
	response to extracellular stimulus	8	0.003
	homeostatic process	15	0.006
	metabolic process	81	0.012
	biological regulation	62	0.83
	regulation of apoptosis	9	0.41
	endocytosis	4	0.3
Cellular constituent	extracellular region	42	<0.0001
	plasma membrane part	28	0.017
	insoluble fraction	13	0.04
	membrane	66	0.16
	cytoplasm	75	0.006
	mitochondrial part	6	0.53
Molecular function	oxidoreductase activity	20	<0.0001
	cofactor binding	10	<0.0001
	lipid binding	12	0.002
	ion binding	47	0.071
	DNA binding	10	1
	signal transducer activity	13	0.99

RAP1GAP (RAP1 GTPase activating protein) is down-regulated in GCA, which reveal six loci influencing plasma levels of liver enzymes in population-based genome-wide association studies (43).

SOX9 (SRY (sex determining region Y)-box 9), transcription factor was up-regulated in GCA, which play cell morphogenesis in different levels and have critical roles in diseases mutation like campomelic dysplasia with autosomal sex reversal (44).

ADA (adenosine deaminase) is down-regulated in GCA response to reactive oxygen species and response to hypoxia cause adenosine deaminase deficiency, partial, severe combined immunodeficiency due to ADA deficiency (45).

AKR1C2 (aldo-ketoreductase family 1) is down-regulated in GCA has function in fatty acid metabolic process that cause obesity, hyperphagia, and developmental delay (46).

Conclusion

The most relationship between genes in GCA and other cancer cells was extracted by comparison of the disease pathways, gene clustering based on GO biological process, functional classes and cellular components. This illustrates correlated functional expression patterns related cancer condition, which might help us to identify new potential drug targets. On top of that, morphologically identical tumors can be distinct in their mutational patterns, signaling-pathway alterations and gene-expression profiles, and, most importantly, in their response to a range of therapies. Therefore, new predictive molecular diagnostics need to be developed and integrated with drug development and clinical-trial design. To this point, there have been a few possible biomarkers for GCA. In this study we introduced some biomarkers that might be used for diagnosis, prognosis and treatment prediction. To unravel the possible role(s) of these proteins in GCA tumorigenesis, further investigations are needed.

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