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ORIGINAL ARTICLE

Gastric cardia adenocarcinoma pathway analysis

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ABSTRACT

Gastric cardia adenocarcinoma (GCA)is one of the few malignancies with unexplained reasons that have increased sharply in developed countries. The purpose of this review was to determine the pathways in GCA to identify new biomarker. So 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 DAVID program to explorer 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 based on the differently expressed genes made up of diseases pathway related 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 so, 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 the 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 have not enough sensitive and specific. So new early stage detection techniques, treatment options and knowledge about the molecular mechanisms of gastric cancer are needed. In this review, 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 resemble esophageal adenocarcinomas (EACs) than gastric noncardia adenocardinoma (GNCA). Because, the cardia anatomically lies between the end of the esophagus and the body of the stomach, genetically reported also, 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 sharply in developed countries in recent years for reasons that are as 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 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). So, the aim of this study was to investigate GCA at the molecular level using pathway analysis, therefore, this makes relatively easy to find candidate marker proteins for GCA. Beside on study based systems provide convenient biomarkers of diagnosing cancer as well as drug targets. In addition to 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 of the functional annotations using UniProt accession numbers (http://www.uniprot.org), a publicly available web-based tool, to search for annotations that are significantly associated to the list of GCA related proteins.

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 lists 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, in turn, help us to identify new potential drug targets; so, in this study have been utilized classification software to 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 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, PLAU, 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 reveres 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 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 that 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 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	GO name	No. of	P-value

categories	genes	
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 the 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, 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, 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, cytoplasm. All these up-regulated expressed genes are functionally related, or members of the same pathway or protein complex are 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 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 SOX-9,was up-regulated in GCA 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 disease pathways, Gene clustering based on GO biological process, functional classes and cellular components illustrate correlated functional expression patterns related cancer condition, which might, in turn, 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. So far, there have been a few possible biomarkers for GCA. In this study introduced some biomarkers that might use for diagnosis, prognosis and treatment prediction. To unravel the possible role(s) of these proteins in GCA tumorigenesis, further investigations are needed.

References

1. Bruckner HW, Morris JC, Mansfield P. Neoplasms of the stomach. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E, III, et al. editors. Cancer Medicine, 5th ed. Ontario, British Columbia: Decker Inc; 2000.

2. Ling XL, Fang DC, Wang RQ, Yang SM, Fang L. Mitochondrial microsatellite instability in gastric cancer and its procancerous lesions. World J Gastroenterol 2004;10:800–803.

3. Sugano K. Gastric cancer: pathogenesis, screening, and treatment. Gastrointest Endosc Clin N Am 2008;18:513–22.

4. di Mario F, Cavallaro LG. Non-invasive tests in gastric diseases. Dig Liver Dis 2008;40:523–30.

5. Opocher E, Santambrogio R, Bianchi P, Cioffi U, De Simone M, Vellini S, Montorsi M. Isolated splenic metastasis from gastric carcinoma: value of CEA and CA 19-9 in early diagnosis: report of two cases. Am J Clin Oncol 2000;23:579–80.

6. Park WS, Oh RR, Park JY, Lee SH, Shin MS, Kim HS, et al. Frequent somatic mutations of the beta-catenin gene in intestinal-type gastric cancer. Cancer Res1999;59: 4257–60.

7. Gleeson CM, Sloan JM, McManus DT, Maxwell P, Arthur K, McGuigan JA, et al. Comparison of p53 and DNA content abnormalities in adenocarcinoma of theoesophagus and gastric cardia. Br J Cancer 1998;77: 277–86.

8. Berx G, Becker KF, Hofler H, van RF. Mutations of the human Ecadherin (CDH1) gene. Hum Mutat 1998;12: 226–37.

9. Park WS, Oh RR, Park JY, Lee JH, Shin MS, Kim HS, et al. Somatic mutations of the trefoil factor family 1 gene in gastric cancer. Gastroenterology 2000;119: 691–98.

10. Lee JH, Han SU, Cho H, Jennings B, Gerrard B. A novel germ line juxta membrane Met mutation in human gastric cancer. Oncogene 2000;19: 4947–53.

11. Suzuki T, Yasui W, Yokozaki H, Naka K, Ishikawa T, Tahara E. Expression of the E2F family inhuman gastrointestinal carcinomas. Int J Cancer 1999; 81:535–38.

12. Cheng XX, Wang ZC, Chen XY, Sun Y, Kong QY, Liu J, et al. Frequent loss of membranous E-cadherin in gastric cancers: a cross-talk with Wnt in determining the fate of beta catenin. Clin Exp Metastasis 2005; 22:85–93.

13. Tytgat GN, Bartelink H, Bernards R, Giaccone G, van Lanschot JJ, Offerhaus GJ, et al. Cancer of the esophagus and gastric cardia: recent advances. Dis Esophagus 2004;17:10–26.

14. Tajima Y, Nakanishi Y, Yoshino T, Kokawa A, Kusano M, Shimoda T. Clinicopathological study of early adenocarcinoma of the gastric cardia:comparison with early adenocarcinoma of the distal stomach and esophagus.Oncology 2001;61: 1–9.

15. Babaei M, Pourfarzi F, Yazdanbod A, Chiniforush MM, Derakhshan MH, MousaviSM, et al. Gastric Cancer in Ardabil, Iran - a Review and Update onCancer Registry Data. Asian Pacific J Cancer Prev 2010;11: 595-99.

16. Gao Y, Hu N, Han XY, Ding T, Giffen C, Goldstein AM, et al. Risk factors foresophagel and gastric cancers in Shanxi Province, China: A case-control study.Cancer Epidemiol 2011;35: 91–99.

17. Anderson WF, Camargo MC, Fraumeni JF Jr, Correa P, Rosenberg PS, Rabkin CS. Age-specific trends in incidence of noncardia gastric cancer in US adults. JAMA 2010;303: 1723–28.

18. Li JY, Taylor PR, Li B, Dawsey S, Wang GQ, Ershow AG, et al. Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. J Natl Cancer Inst 1993;85: 1492–98.

19. Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H, Gunter EW, et al. Prospective study of serum selenium levels and incident esophageal and gastric cancers. J Natl Cancer Inst 2000;92: 1753–63.

20. Wei W, Abnet CC, Qiao YL, Dawsey SM, Dong ZW, Sun XD, et al. Prospective study of serum selenium concentration and esophageal and gastric cardiacancer, heart disease, stroke, and total death. Am J Clin Nutr 2004;79: 80–85.

21. Gao Y, Hu N, Han X, Giffen C, Ding T, Goldstein A, et al. Family history of cancer and risk for esophageal and gastric cancer in Shanxi, China. BMC Cancer 2009;9:269.

22. Sipponen P. Helicobacter pylori: a cohort phenomenon. Am J SurgPathol 1995;1: S30-36.

23. Tran GD, Sun XD, Abnet CC, Fan JH, Dawsey SM, Dong ZW, et al. Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. Int J Cancer 2005;113: 456–63.

24. Kamangar F, Qiao YL, Blaser MJ, Sun XD, Katki H, Fan JH, et al. Helicobacter pylori and oesophageal and gastric cancers in a prospective study in China.Br J Cancer 2007;96: 172–76.

25. Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, et al. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. Nat Genet 2010;42: 764–67.

26. Wang G, Hu N, Yang HH, Wang L, Su H, Wang C, et al. Comparison of global gene expression of gastric cardia and noncardia cancers from a high-risk population in China. PLoS ONE 2013;8: 63826.

27. Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44–57.

28. Bergmann S, Ihmels J, Barkai N. Similarities and differences in genome-wide expression data of six organisms. PLoS Biol 2004; 2: 9.

29. Stuart JM, Segal E, Koller D, Kim SK. A gene coexpression network for global discovery of conserved genetic modules. Science 2003; 302: 249–55.

30. Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, et al. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. Nat Genet 2010;42: 764–67.

31. Derakhshan MH, Yazdanbod A, Sadjadi AR, Shokoohi B, McColl KE, Malekzadeh R. High incidence of adenocarcinoma arising from the right side of the gastric cardia in NW Iran. Gut 2004;53, 1262-66.

32. Malekzadeh R, Sotoudeh V, Derakhshan MH, Mikaeli J, Yazdanbod A, Merat S, et al. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the north west of Iran. J Clin Pathol 2004;57:37-42.

33. Jinawath N, Furukawa Y, Hasegawa S, Li M, Tsunoda T, Satoh S, et al. Comparison of gene-expression profiles between diffuse- and intestinal-type gastric cancers using a genome-wide cDNA microarray. Oncogene 2004;23: 6830–44.

34. Boussioutas A, Li H, Liu J, Waring P, Lade S, Holloway AJ, et al. Distinctive patterns of gene expression in premalignant gastric mucosa and gastric cancer. Cancer Res 2003;63: 2569–77.

35. Chen X, Leung SY, Yuen ST, Chu KM, Ji J, Li R, et al. Variation in gene expression patterns in human gastric cancers. Mol Biol Cell 2003;14: 3208–15.

36. Rio FT, Lavoie J, Hamel N, Geyer FC, Kushner YB, Novak DJ, et al. HomozygousBUB1B mutation and susceptibility to gastrointestinal neoplasia. N Engl J Med 2010;363: 2628–37.

37. Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet 2011;43: 34–41.

38. Hasegawa S, Furukawa Y, Li M, Satoh S, Kato T, Watanabe T, et al. Genome-wide analysis of gene expression in intestinal-type gastric cancers using acomplementary DNA microarray representing 23,040 genes. Cancer Res 2002;62:7012–17.

39. Kim B, Bang S, Lee S, Kim S, Jung Y, Lee C, et al. Expression profiling and subtype-specific expression of stomach cancer. Cancer Res 2003;63: 8248–55.

40. Lee M, Liu M, Lee C. Gene therapy for prostate cancer targeting transcription factor E2F/DRTF. Int J Oncol 1997;10:729-34.

41. Rabbani F, Richon VM, Orlow I, Lu ML, Drobnjak M, Dudas M, et al. Prognostic significance of transcription factor E2F-1 in bladder cancer: genotypic and phenotypic characterization. J Natl Cancer Inst 1999; 91: 874-81.

42. Tsukasaki K, Miller CW, Greenspun E, Eshaghian S, Kawabata H, FujimotoT, et al. Mutations in the mitotic check point gene, MAD1L1, in human cancers. Oncogene 2001;20: 3301-305.

43. Oxana M. Down regulation of Rap1GAP in human tumor cells alters cell/matrix and cell/cell adhesion. Mol Cell Biol 2010; 30: 3262–74.

44. Giordano J, Prior HM, Bamforth JS, Walter MA. Genetic study of SOX9 in a case of campomelic dysplasia. Am J Med Genet 2001;98:176-81.

45. Buchbinder D, Puthenveetil G, Soni A, Hsieh L, Nugent D, Church JA. Newborn screening for severe combined immunodeficiency: an opportunity for intervention. J Perinatol 2013;33:657-58.

46. Li Z, He X, Xing S, Ni J, Zhang W, Xu X, et al. Overexpression of Aldo-keto reductase family 1 B10 protein in ductal carcinoma in situ of the breast correlates with HER2 positivity. Cancer Biomark 2013;13:181-92.