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In silico analysis of stomach lineage specific gene set expression pattern in gastric cancer

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ABSTRACT

Stomach lineage specific gene products act as a protective barrier in the normal stomach and their expression maintains the normal physiological processes, cellular integrity and morphology of the gastric wall. However, the regulation of stomach lineage specific genes in gastric cancer (GC) is far less clear. In the present study, we sought to investigate the role and regulation of stomach lineage specific gene set (SLSGS) in GC. SLSGS was identified by comparing the mRNA expression profiles of normal stomach tissue with other organ tissue. The obtained SLSGS was found to be under expressed in gastric tumors. Functional annotation analysis revealed that the SLSGS was enriched for digestive function and gastric epithelial maintenance. Employing a single sample prediction method across GC mRNA expression profiles identified the under expression of SLSGS in proliferative type and invasive type gastric tumors compared to the metabolic type gastric tumors. Integrative pathway activation prediction analysis revealed a close association between estrogen-α signaling and SLSGS expression pattern in GC. Elevated expression of SLSGS in GC is associated with an overall increase in the survival of GC patients. In conclusion, our results highlight that estrogen mediated regulation of SLSGS in gastric tumor is a molecular predictor of metabolic type GC and prognostic factor in GC.

1. Introduction

Stomach lineage specific gene products are mainly involved in gastric mucosal regeneration and help maintain the integrity of the gastric mucosal epithelium. Under or decreased expression of stomach lineage specific genes in stomach tissue positively correlates with the incidence of GC [1–3]. GC arises from inflammation and is preceded by a lengthy precancerous process, developing *via* multiple sequential steps [4]. GC ranks fourth in terms of prevalence and second in terms of mortality with a projected 6,50,000 deaths annually [5]. GCs are highly heterogeneous with distinct pathological patterns and clinical behaviors [6]. Most cases of GC are diagnosed at late stages with a five year survival rate of 24%. However, if GC is diagnosed at early stages, the five-year survival rate is about 61% [7]. Lauren classification is the traditional method for classifying GCs into histological subtypes according to the structural features, histological appearances of the cells and the level of mucus [8]. The current treatment methods for GC mainly depend on the Lauren classification based pathological staging of the disease and TNM staging system [9]. Due to heterogeneity, treatment methods and prognosis of individual GCs based on the clinical and pathological classification vary among GC patients. As an alternate to the pathological staging system in conventional treatment methods, well-conducted genomic studies have stimulated changes in surgical decision-making and therapy options. High throughput genomic technologies have been used to identify gene expression based GC molecular sub-types and prognostic markers for improved diagnosis, prognosis and therapeutic options [10–13]. Recent reports have shown various gene expression signatures that can be used to predict clinical endpoints, such as patient survival and therapy response of various cancers [14,15]. Fewer studies also identified the gene signatures/gene sets associated with GC subtype and survival [16–18]. However, investigation of the SLSGS in GC remains unexplored till date. Understanding the expression and molecular regulation mechanisms of stomach lineage specific genes in gastric carcinogenesis may allow the use of effective targeted therapies. In this study, we have identified the SLSGS and investigated the role, regulation and prognostic associations of the gene set in GC.

Abbreviations: SLSGS, stomach lineage specific gene set; GC, gastric cancer; GCs, Gastric cancers; MGC, metabolic gastric cancer; IGC, invasive gastric cancer; PGC, proliferative gastric cancer; DWD, distance weighted discrimination; SSP, single sample prediction; IPAP, *in silico* pathway activation prediction; ER-α, Estrogen-α; GSEA, gene set enrichment analysis; DAVID, database for annotation, visualization, and integrated discovery; ROC, receiver operating characteristic; GO, gene ontology; mSigDB, molecular signature database; GLSGS, stomach lineage specific gene set.

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2. Materials and methods

2.1. Data set collection

Microarray mRNA expression profiles used in the study were collected from Gene Expression Omnibus (GEO) [19,20]. The expression profiles analyzed in the study were normalized and $\log_2$ transformed. The platform specific probes were mapped to gene symbols with appropriate annotation files. Expression values of genes with multiple probes were considered for analysis after averaging. Differential expression analysis among stomach tissues and other organ tissues were done with BRB array tools. Gene expression changes above 2.5-fold with p-value less than 0.05 were considered. Supervised clustering analysis was done using Dchip clustering software.

2.2. Distance Weighted Discrimination

Distance Weighted Discrimination (DWD) was performed using a DWDSSP 1.0 tool, in order to correct the systemic biases from different microarray expression data sets in a pair wise fashion by combining the two data sets. In the final step, each microarray experiment was normalized such that each column/experimental sample was standardized to N (0,1), and each row/gene was median centered [21,22].

2.3. Gene set enrichment, gene ontology, disease ontology and co-expression analysis

SLSGS expression values were extracted from normalized and $\log_2$ transformed gastric tumor profiles. Gene set enrichment analysis (GSEA) was performed for SLSGS across multiple cohorts of GC mRNA expression profiles. The number of phenotype permutations involved in the nominal p-value calculation was 1000. A database for annotation, visualization, and integrated discovery (DAVID) functional annotation tool was used to identify SLSGS in gene ontology (GO) biological process category and determine the percentage occurrence of SLSGS for each biological process [23,24]. Disease features ontology-based overview system (Gendoo) was used to identify disease ontology annotation of SLSGS [25,26]. Co-expression analysis of SLSGS in the normal tissue panel and different types of tumors were performed using the Topp cluster tool [27].

2.4. Single Sample Prediction

The Single Sample Predictor (SSP) is a nearest centroid based method for classifying an individual sample according to its nearest centroid as determined by Spearman correlation [22,28]. To create each GC subtype (proliferative GC, metabolic GC and invasive GC) we averaged the gene expression profiles of GC samples clearly assigned to each subtype. DWD integrated training and test data sets were used for SSP analysis. SSP was performed with DWDSSP 1.0 tool to predict molecular subtypes of GC. Using similar methods, the SSP was applied to four independent cohorts of GC mRNA expression profiles.

2.5. In silico pathway activation prediction

Gene sets used for *in silico* pathway activation prediction (IPAP) analysis were obtained from molecular signature database (mSigDB) [29]. The data sets used for the gene set activation prediction were Z-normalized as described earlier [30]. The expression values of the gene sets were additively combined to get an "activation score". Detailed descriptions about the signatures used in the study are provided in the supplementary material (Table S6). SLSGS expression with signaling pathway correlation was analyzed using 'R' bio-conductor. Variable graph was plotted using MedCalc 12.7.0.0 statistical platform.

2.6. Survival analysis

We considered overall survival information of the GC patients for predicting the association between gene expression and patient survival (GSE4007). Based on the cox coefficient values, patients were classified into low and high risk groups [31]. Cox regression coefficient value for each gene and its relation to the survival was calculated. Survival curve was plotted with 'R' bio-conductor.

3. Results

3.1. Identification of stomach lineage specific gene set

A principal component analysis showed the effect of DWD integration on the first two principal components. DWD removed the data set bias very efficiently (Fig. 1A). SLSGS was identified from the DWD integrated (GSE3526 and GSE7307) normal tissue panel mRNA expression profiles by comparing 12 stomach tissues with 363 other organ tissues. Fifty stomach lineage specific genes with significant fold change were identified (Fig. 1B). Further investigation of SLSGS in two independent cohorts of mRNA expression profile comprising various normal tissues of the body confirmed the stomach tissue specific expression of SLSGS (Fig. 1C, Table S1). Immuno histochemical data obtained from human protein atlas database also supported the stomach tissue specific expression of SLSGS (Fig. 1D, Table S2).

3.2. Analysis of SLSGS expression pattern in GC

The SLSGS expression pattern was investigated in eight independent cohorts of GC mRNA expression profiles comprising 263 normal and 407 tumor gastric tissues (Table S3). 33 out of 50 genes were strongly down-regulated in gastric tumor compared to normal gastric tissue as inferred from the DWD integrated GC mRNA expression profiles (GSE13911 and GSE19826) (Fig. 2A, Figure. S1). Since the expression level changes of the remaining 17 genes were statistically insignificant ($p > 0.05$) the remaining 33 genes (Table S4) were considered for further analysis. GSEA was performed for the SLSGS in six independent cohorts of GC mRNA expression profiles and highlighted enrichment of SLSGS in normal gastric tissue (Fig. 2B). Fold change values of SLSGS expression across normal and gastric tumor profiles identified a distinct down regulation of these genes in gastric tumors (Table S5).

3.3. Gene ontology, disease ontology and co-expression analysis of SLSGS

Subsets of the SLSGS involved in digestion, immune system, gastric epithelial maintenance, ion transport and digestive system were highlighted by means of a pie chart. GO analysis of SLSGS also supported our inferences drawn from annotation indicating the enrichment of stomach specific biological processes (Fig. 2C). Disease ontology annotation demonstrated the involvement of SLSGS in various diseases and a strong association between SLSGS and gastrointestinal cancer (Figure. S2). Normal tissue atlas based co-expression network of SLSGS in various normal tissues showed that significant co-expression occurred only in normal stomach tissues (Figure. S3). Co-expression analysis of SLSGS in various tumor types indicated a significant co-expression in gastrointestinal tumors as compared to other types of tumors (Fig. 2D).

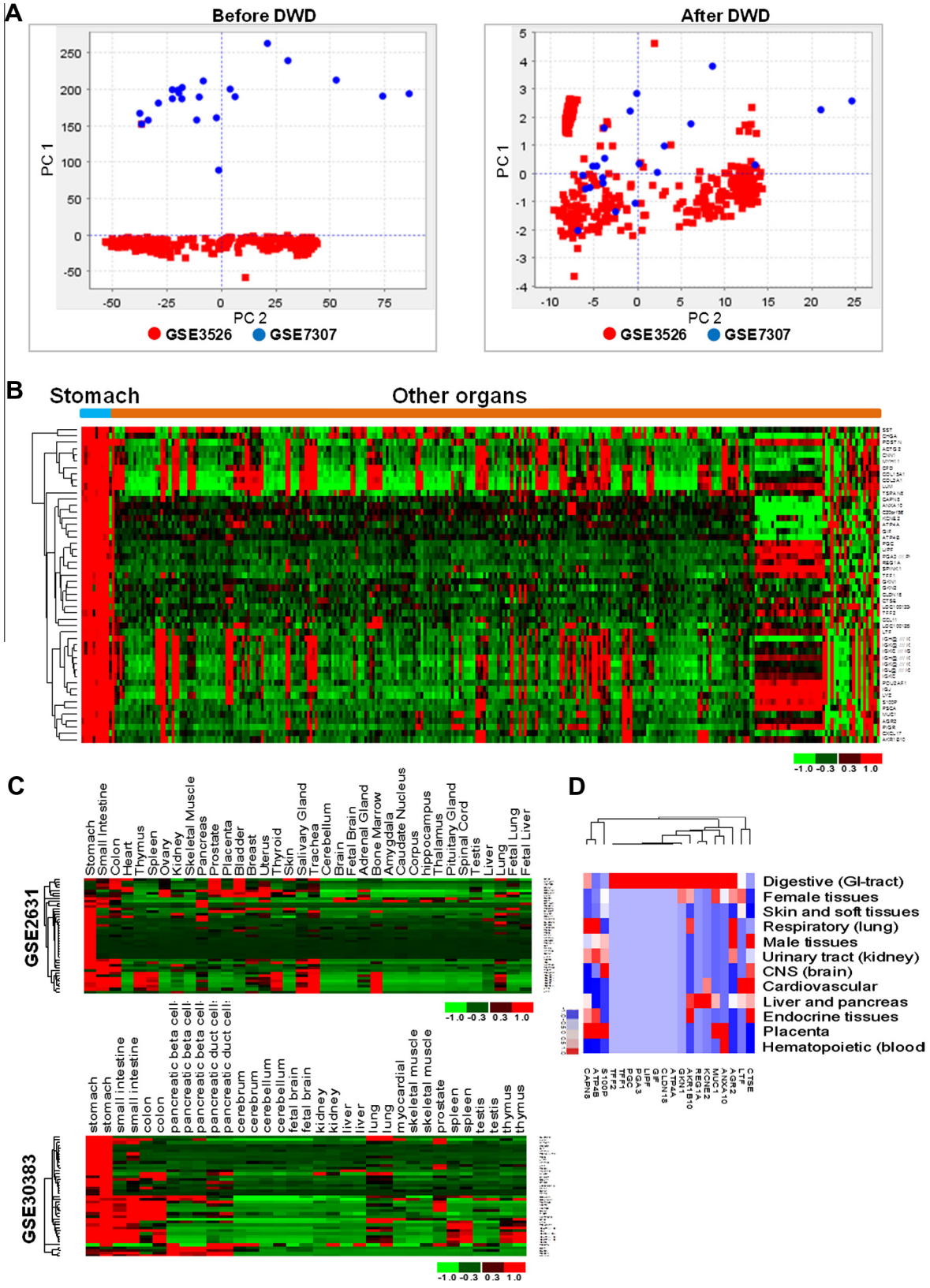

Fig. 1. Identification of stomach lineage specific gene set. (A) Principal components of the effect of DWD mediated integration of two independent cohorts of normal tissue panel mRNA expression profiles. (B) Expression pattern of SLSGS in DWD integrated normal tissue mRNA expression profile. (C) The identified SLSGS showing an elevated expression in stomach tissue when compared to the other organ tissues in two different cohorts. (D) Immuno histochemical staining pattern of SLSGS showing an elevated expression in stomach tissue in comparison with other organ tissues.

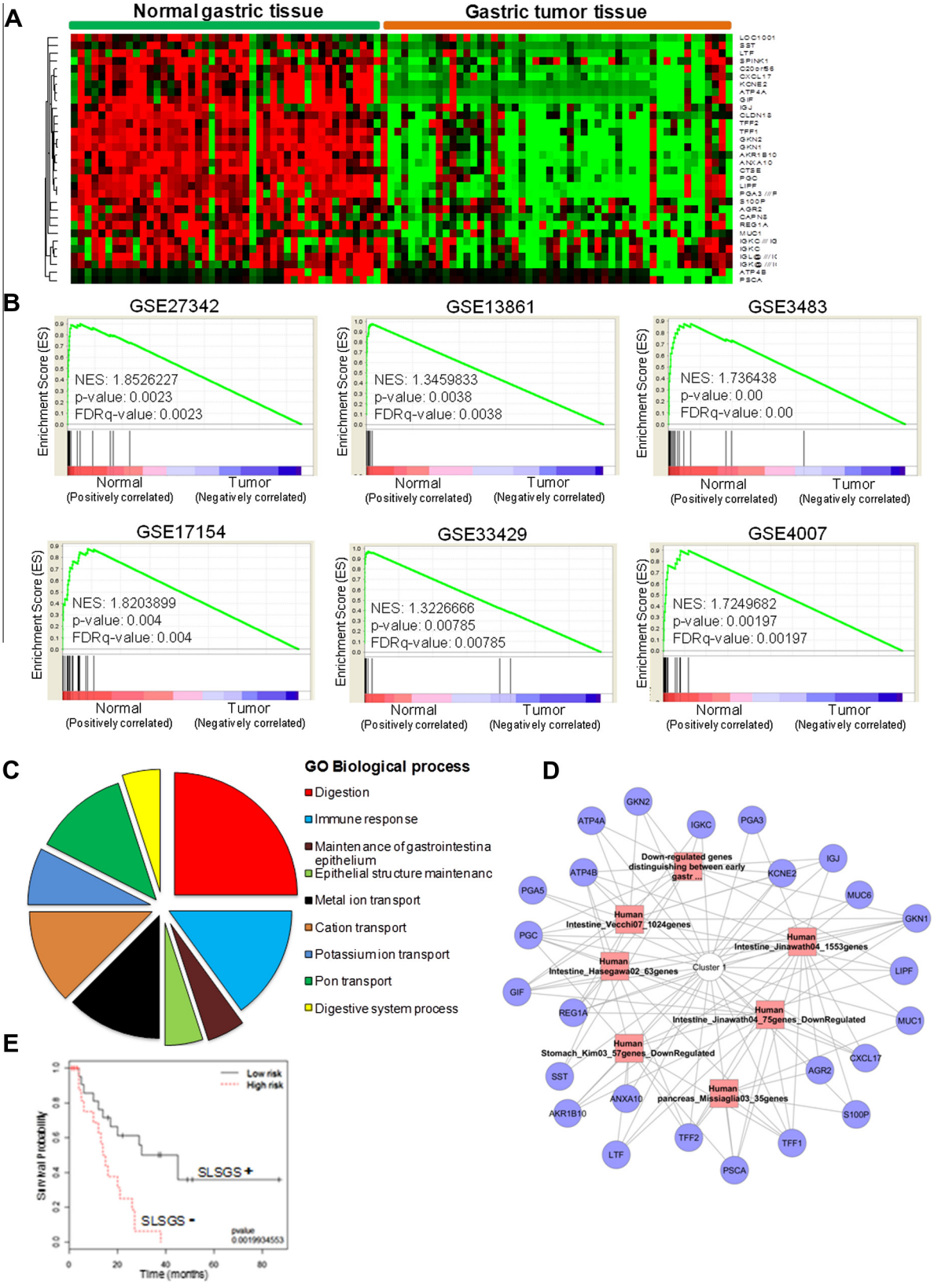

Fig. 2. Functional annotation and investigation of SLSGS expression pattern. (A) Heatmap showing the expression pattern of SLSGS in DWD integrated GC mRNA expression profile. Comparative analysis of SLSGS expression between normal and tumorous gastric tissues reveals decreased SLSGS expression in gastric tumors. (B) GSEA showing an enrichment of SLSGS in normal gastric tissue in six different cohorts of GC mRNA expression profiles. (C) Pie chart summary of GO biological processes showing an involvement of SLSGS in gastric mucosal maintenance and digestive function. (D) Co-expression network of SLSGS in various types of tumors showing significant association between SLSGS expression and gastro intestinal tumors. (E) Cox regression coefficient based survival analysis of SLSGS in GC patients. Low risk represents an elevated SLSGS expression while high risk represents decreased SLSGS expression in GC. Elevated expression of SLSGS was associated with better clinical outcome in gastric tumors.

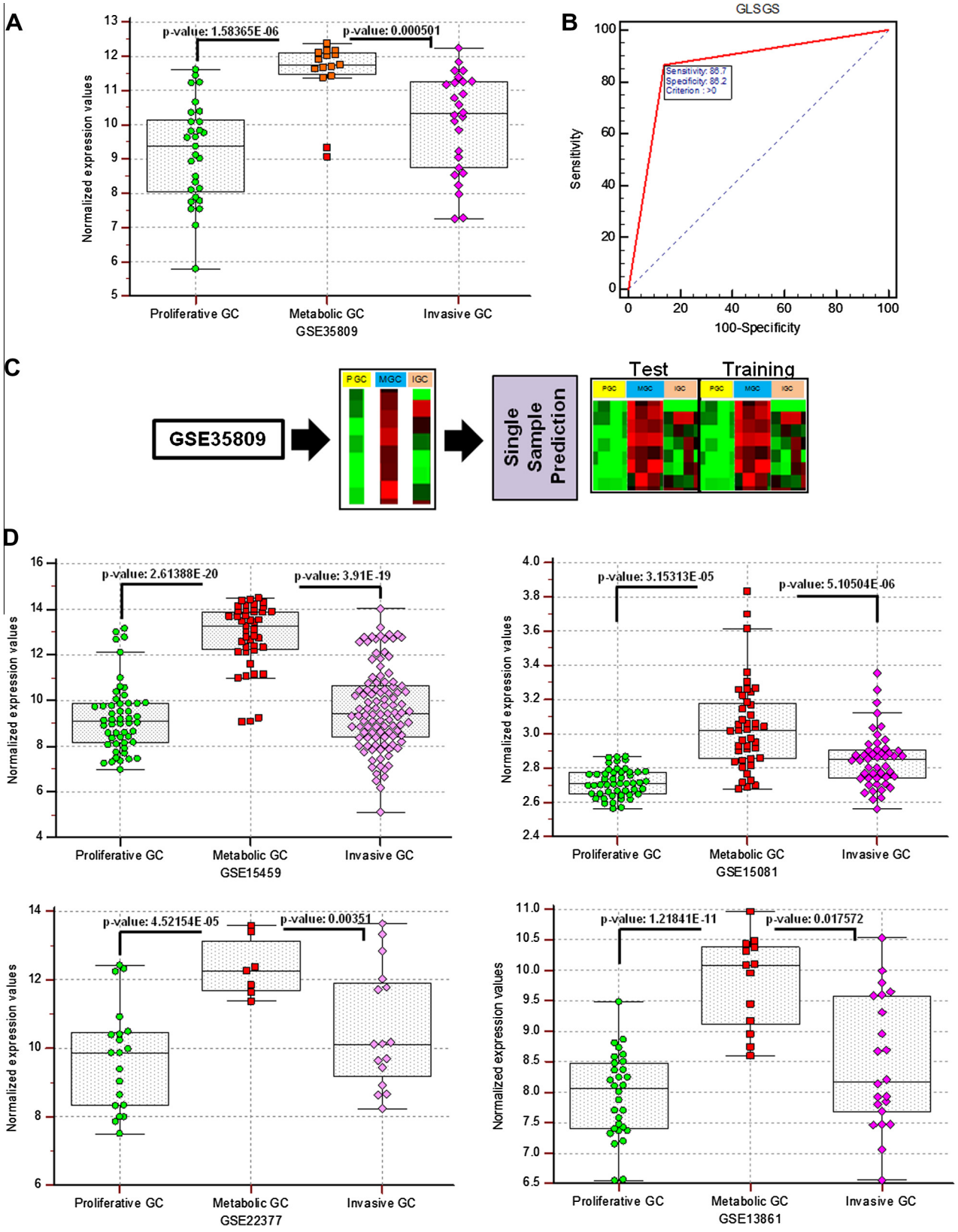

Fig. 3. Analysis of SLSGS expression pattern in molecular subtypes of GC. (A) The expression pattern of SLSGS among proliferative, metabolic and invasive type gastric tumors reveals the elevated SLSGS expression in metabolic gastric tumors. (B) Sensitivity and specificity analysis of SLSGS in GC mRNA expression profile GSE35809. (C) Schematic representation of single sample prediction method employed for molecular subtype prediction. (D) Expression pattern of SLSGS in four independent cohorts of GC mRNA profile showing an elevated expression of SLSGS in metabolic gastric tumors.

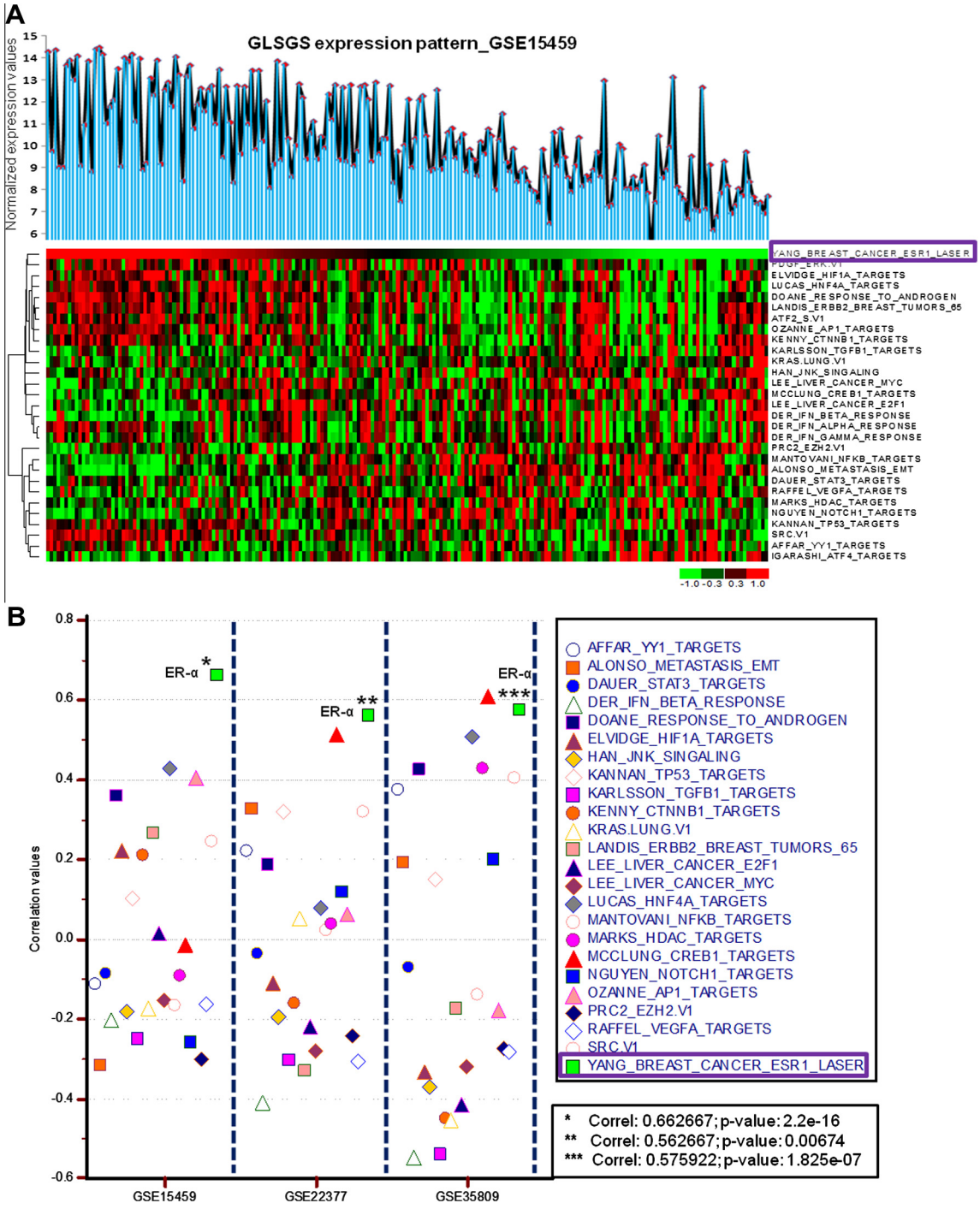

Fig. 4. Identification of signaling processes associated with SLSGS expression in GC. (A) Regulation of multiple signaling pathways in 200 samples of GC mRNA expression profile represented as a heat map. Multiple signaling pathways were analyzed and the comprehensive activation (red) and inactivation (green) pattern across samples is depicted. The black color line graph represents the corresponding expression pattern of SLSGS in same GC mRNA expression profile. (B) Variable graph representing the association of various signaling pathways with the SLSGS expression pattern in three different GC mRNA expression profiles reveal a correlation between SLSGS and estrogen signaling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Exploration of prognostic association of SLSGS in GC

A prognostic association of SLSGS was investigated in GC mRNA expression profile (GSE4007). Survival analysis of SLSGS identified that the low-risk group was associated with elevated expression of SLSGS while the high risk group displayed decreased expression of SLSGS (Fig. 2E). Thus, survival analysis strongly suggests that elevated expression of SLSGS is associated with better overall survival of GC patients.

3.5. Investigation of SLSGS expression pattern in molecular subtypes of GC

The SLSGS expression pattern was investigated in a GC mRNA profile (GSE35809) comprised of 15 metabolic type, 29 proliferative type and 26 invasive type gastric tumors. Elevated expression of SLSGS was observed in metabolic type GC (MGC) with significant fold change compared to proliferative and invasive type gastric tumors (Fig. 3A). Receiver operating characteristic (ROC) analysis of

SLSGS showed 86.7% sensitivity and 86.2% specificity in predicting metabolic type gastric tumors (Fig. 3B). The SLSGS expression pattern was analyzed in subtype predicted four independent cohorts of GC mRNA expression profile comprised of 455 GC samples. Analysis supported that elevated expression of SLSGS was significantly associated with metabolic type gastric tumors (Fig. 3D).

3.6. Identification of possible regulators of SLSGS in GC

In order to explore the possible regulators of SLSGS in GC, gene signature based IPAP was employed in the mRNA expression profiles of three GC cohorts comprised of 313 GC samples. Twenty-nine oncogenic signatures comprising of both up and down regulated genes representing various oncogenic pathways collected from mSigDB were used for this analysis (Table S6). Three GC profiles (GSE15459, GSE35809 and GSE22377) were analyzed to score the activation of pathway signatures using gene set based IPAP approach. Hierarchical supervised clustering was performed for signature based activation scores (Fig. 4A, Figure. S4). Pattern simulation analysis was used to identify the correlation between SLSGS and oncogenic signaling pathways. Correlation values were calculated for SLSGS with all 29 signatures. Signature based IPAP method identified a significant positive association between SLSGS gene expression and ER-α signaling in all three gastric tumor cohorts (Fig. 4B, Table S7).

4. Discussion

SLSGS mRNA expression pattern and immuno histochemical scores from human protein atlas highlighted the stomach lineage specific expression of SLSGS. Few members of SLSGS have been already reported as digestive enzymes and stomach mucosal secreted proteins, which are involved in mucosal repair and suppression of the stomach tumor formation [1,32–34]. Analysis of SLSGS in GC mRNA expression profiles revealed that expression of SLSGS was down regulated in GC tissue compared to the normal gastric tissue. Epigenetic and oncogenic signaling pathways are the major factors for the decreased expression of stomach specific genes in gastric tumors. In close agreement with previous studies, our study also identified the down regulation pattern of stomach lineage specific genes in GC [35,36].

Functional annotation analysis of SLSGS, confirmed the gastrointestinal tissue specific biological functions. Functional annotation also revealed the essentiality of SLSGS expression in stomach epithelium maintenance. Disease ontology analysis identified that a few genes of SLSGS were also involved in other cancer types [37–39]. However, SLSGS was majorly involved in gastrointestinal tumors [40,41]. Co-expression analysis of SLSGS in normal tissue types identified stomach tissue specific co-expression pattern supporting our earlier findings in terms of tissue specificity. Similarly, SLSGS co-expression in various tumor types identified the distinct co-expression pattern in gastrointestinal tumors. Survival analysis of SLSGS in GC showed SLSGS has prognostic value in patients with GC. Analysis revealed that elevated expression of SLSGS was associated with better overall survival of GC patients. Further investigation with different cohorts is required to validate the identified association between SLSGS expression and survival of GC patients. The cumulative results of functional annotation, disease annotation, co-expression and survival analysis support the stomach specific protective role of SLSGS.

Elevated expression of SLSGS was largely specific to MGC as compared to the PGC and IGC. SSP method applied to four independent cohorts of the GC mRNA expression profile also revealed the elevated expression of SLSGS in MGC. Recent molecular sub typing of GC identified that metabolic subtype of GC is partially associated with the characteristic of normal gastric mucosal gene expression [11]. Furthermore, GO based biological process annotation identified the enrichment of digestive function [11] in these genes.

Unravelling the factors regulating SLSGS by signaling based pathway activation prediction revealed closer positive association of ER-α signaling with elevated expression of SLSGS in GC. IPAP analysis identified a strong positive association between ER-α and elevated SLSGS expression in three independent GC cohorts. Previous studies have also suggested a relationship between ER-α signaling and GC. Significant amounts of estrogen receptor were expressed in gastric mucosa while expression levels were significantly lower in GC. Additionally, estrogen has also been associated with protection against GC in women [42,43]. Few genes of SLSGS were already reported as estrogen responsive in different cancer types [44,45]. Concordant with the expression pattern of estrogen, SLSGS expression was also enriched in normal stomach phenotype and down regulated in GC clearly suggesting an estrogen mediated regulation.

The cumulative findings of study data strongly suggest that, SLSGS expression is stomach tissue specific and SLSGS is a molecular predictor of metabolic type GC. Elevated expression of SLSGS in GC shows strong positive association with ER-α signaling and is associated with better survival of GC patients. Additional mechanisms involved in the regulation of SLSGS in GC tissue require further investigation. These genes with expression patterns capable of distinguishing GCs of molecular subtype will provide useful information towards developing a gene expression-based grading system for GC and might provide new opportunities for therapeutic intervention.

Competing interests

The authors of this paper declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.007.

References

[1] G.S. Wang, W.W. Cui, B.Y. Wu, et al., Gene expression profile of human normal gastrointestinal tract tissues: bioinformatic study, Zhongguo Ying Yong Sheng Li Xue Za Zhi 24 (2008) 334–337.
[2] W. Mao, J. Chen, T.L. Peng, et al., Downregulation of gastrokine-1 in gastric cancer tissues and restoration of its expression induced gastric cancer cells to apoptosis, J. Exp. Clin. Cancer Res. 31 (2012) 49.
[3] S.F. Moss, J.W. Lee, E. Sabo, et al., Decreased expression of gastrokine 1 and the trefoil factor interacting protein TFIZ1/GKN2 in gastric cancer: influence of tumor histology and relationship to prognosis, Clin. Cancer Res. 14 (2008) 4161–4167.
[4] R.C. Orlando, Mechanisms of epithelial injury and inflammation in gastrointestinal diseases, Rev. Gastroenterol. Disord. 2 (Suppl 2) (2002) S2–S8.
[5] L. Zheng, L. Wang, J. Ajani, et al., Molecular basis of gastric cancer development and progression, Gastric Cancer 7 (2004) 61–77.
[6] C.H. Ooi, T. Ivanova, J. Wu, et al., Oncogenic pathway combinations predict clinical prognosis in gastric cancer, PLoS Genet. 5 (2009) e1000676.
[7] Cancer Facts & Figures 2008, American Cancer Society's publication.
[8] P. Lauren, The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification, Acta Pathol. Microbiol. Scand. 64 (1965) 31–49.

[9] M. Werner, K.F. Becker, G. Keller, et al., Gastric adenocarcinoma: pathomorphology and molecular pathology, J. Cancer Res. Clin. Oncol. 127 (2001) 207–216.
[10] B.J. Dicken, D.L. Bigam, C. Cass, et al., Gastric adenocarcinoma: review and considerations for future directions, Ann. Surg. 241 (2005) 27–39.
[11] Z. Lei, I.B. Tan, K. Das, et al., Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil, Gastroenterology 145 (2013) 554–565.
[12] I.B. Tan, T. Ivanova, K.H. Lim, et al., Intrinsic subtypes of gastric cancer, based on gene expression pattern, predict survival and respond differently to chemotherapy, Gastroenterology 141 (2011) 476–485. 485 e1–11.
[13] K.G. Norsett, A. Laegreid, H. Midelfart, et al., Gene expression based classification of gastric carcinoma, Cancer Lett. 210 (2004) 227–237.
[14] X. Zhao, E.A. Rodland, T. Sorlie, et al., Combining gene signatures improves prediction of breast cancer survival, PLoS ONE 6 (2011) e17845.
[15] X. Chen, S. Xu, M. McClelland, et al., An accurate prostate cancer prognosticator using a seven-gene signature plus Gleason score and taking cell type heterogeneity into account, PLoS ONE 7 (2012) e45178.
[16] J. Shen, J. Wei, H. Wang, et al., A three-gene signature as potential predictive biomarker for irinotecan sensitivity in gastric cancer, J. Transl. Med. 11 (2013) 73.
[17] Z. Yan, W. Xu, Y. Xiong, et al., Highly accurate two-gene signature for gastric cancer, Med. Oncol. 30 (2013) 584.
[18] Y.L. Yap, X.W. Zhang, D. Smith, et al., Molecular gene expression signature patterns for gastric cancer diagnosis, Comput. Biol. Chem. 31 (2007) 275–287.
[19] T. Barrett, S.E. Wilhite, P. Ledoux, et al., NCBI GEO: archive for functional genomics data sets–update, Nucleic Acids Res. 41 (2013) D991–D995.
[20] R. Edgar, M. Domrachev, A.E. Lash, Gene Expression Omnibus: NCBI gene expression and hybridization array data repository, Nucleic Acids Res. 30 (2002) 207–210.
[21] M. Benito, J. Parker, Q. Du, et al., Adjustment of systematic microarray data biases, Bioinformatics 20 (2004) 105–114.
[22] Z. Hu, C. Fan, D.S. Oh, et al., The molecular portraits of breast tumors are conserved across microarray platforms, BMC Genomics 7 (2006) 96.
[23] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[24] W. Huang da, B.T. Sherman, R.A. Lempicki, Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists, Nucleic Acids Res. 37 (2009) 1–13.
[25] T. Nakazato, H. Bono, H. Matsuda, et al., Gendoo: functional profiling of gene and disease features using MeSH vocabulary, Nucleic Acids Res. 37 (2009) W166–W169.
[26] T. Nakazato, T. Takinaka, H. Mizuguchi, et al., BioCompass: a novel functional inference tool that utilizes MeSH hierarchy to analyze groups of genes, In Silico Biol. 8 (2008) 53–61.
[27] V. Kaimal, E.E. Bardes, S.C. Tabar, et al., ToppCluster: a multiple gene list feature analyzer for comparative enrichment clustering and network-based dissection of biological systems, Nucleic Acids Res. 38 (2010) W96–W102.
[28] B. Haibe-Kains, C. Desmedt, S. Loi, et al., A three-gene model to robustly identify breast cancer molecular subtypes, J. Natl. Cancer Inst. 104 (2012) 311–325.
[29] A. Subramanian, P. Tamayo, V.K. Mootha, et al., Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. USA 102 (2005) 15545–15550.
[30] J. Barretina, G. Caponigro, N. Stransky, et al., The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity, Nature 483 (2012) 603–607.
[31] H.Y. Chen, S.L. Yu, C.H. Chen, et al., A five-gene signature and clinical outcome in non-small-cell lung cancer, N. Engl. J. Med. 356 (2007) 11–20.
[32] M.H. Ruchaud-Sparagano, B.R. Westley, F.E. May, The trefoil protein TFF1 is bound to MUC5AC in human gastric mucosa, Cell. Mol. Life Sci. 61 (2004) 1946–1954.
[33] D. Basso, A.L. Stefani, N. Gallo, et al., Biochemical markers of gastric functioning, Recenti Prog. Med. 90 (1999) 342–346.
[34] T.R. Menheniott, B. Kurklu, A.S. Giraud, Gastrokines: stomach-specific proteins with putative homeostatic and tumor suppressor roles, Am. J. Physiol. Gastrointest. Liver Physiol. 304 (2013) G109–G121.
[35] M. Vecchi, P. Nuciforo, S. Romagnoli, et al., Gene expression analysis of early and advanced gastric cancers, Oncogene 26 (2007) 4284–4294.
[36] N. Jinawath, Y. Furukawa, S. Hasegawa, et al., Comparison of gene-expression profiles between diffuse- and intestinal-type gastric cancers using a genome-wide cDNA microarray, Oncogene 23 (2004) 6830–6844.
[37] E. Buache, N. Etique, F. Alpy, et al., Deficiency in trefoil factor 1 (TFF1) increases tumorigenicity of human breast cancer cells and mammary tumor development in TFF1-knockout mice, Oncogene 30 (2011) 3261–3273.
[38] E.S. Kropotova, R.A. Tychko, O.L. Zinov'eva, et al., Downregulation of AKR1B10 gene expression in colorectal cancer, Mol. Biol. (Mosk) 44 (2010) 243–250.
[39] S.A. Tomlins, D.R. Rhodes, J. Yu, et al., The role of SPINK1 in ETS rearrangement-negative prostate cancers, Cancer Cell 13 (2008) 519–528.
[40] G. Wang, N. Hu, H.H. Yang, et al., Comparison of global gene expression of gastric cardia and noncardia cancers from a high-risk population in china, PLoS ONE 8 (2013) e63826.
[41] J.M. Kim, H.Y. Sohn, S.Y. Yoon, et al., Identification of gastric cancer-related genes using a cDNA microarray containing novel expressed sequence tags expressed in gastric cancer cells, Clin. Cancer Res. 11 (2005) 473–482.
[42] E. Chandanos, J. Lagergren, Oestrogen and the enigmatic male predominance of gastric cancer, Eur. J. Cancer 44 (2008) 2397–2403.
[43] S. Singh, R. Poulsom, N.A. Wright, et al., Differential expression of oestrogen receptor and oestrogen inducible genes in gastric mucosa and cancer, Gut 40 (1997) 516–520.
[44] J.M. Sun, V.A. Spencer, L. Li, et al., Estrogen regulation of trefoil factor 1 expression by estrogen receptor alpha and Sp proteins, Exp. Cell Res. 302 (2005) 96–107.
[45] K.E. Vanderlaag, S. Hudak, L. Bald, et al., Anterior gradient-2 plays a critical role in breast cancer cell growth and survival by modulating cyclin D1, estrogen receptor-alpha and survivin, Breast Cancer Res. 12 (2010) R32.