MINI-REVIEW

Serum Protein and Genetic Tumor Markers of Gastric Carcinoma

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Abstract

   The high incidence of gastric cancer and consequent mortality pose severe threats to human health. Early screening, diagnosis and treatment are the key to improve the prognosis of the patients with gastric cancer. Gastroscopy with biopsy is an efficient method for the diagnosis of early gastric cancer, but the associated discomfort and high cost make it difficult to be a routine method for screening gastric cancer. Serum tumor marker assay is a simple and practical method for detection of gastric cancer, but it is limited by poor sensitivity and specificity. Therefore, people have been looking for novel serum markers of gastric cancer in recent years. Here we review the novel serum tumor markers of gastric cancer and their diagnostic significance, focusing on the discoveries from serum proteomics analyses and epigenetics researches.

Keywords: Gastric cancer - serum tumor markers - screening and diagnosis

Introduction

   Gastric cancer is one of the most frequent malignant tumors with high mortality due to the lack of convenient methods for early screening and diagnosis in clinical practice. Gastroscopy with biopsy is currently an efficient method for the diagnosis of gastric cancer, but it is not appropriate for screening gastric cancer because of its discomfort and high cost. The detection of serum tumor markers is simple in cancer screening and diagnosis, but it is difficult to be a conventional method for gastric cancer screening for its poor sensitivity and specificity (Sturgeon et al., 2010), only valuable in the prognostic evaluation of patients with gastric cancer (Dilege et al., 2010; Duraker et al., 2001; Emoto et al., 2012; Li et al., 2011; Ychou et al., 2000). Recently, a lot of studies were performed in serum proteomics and epigenetics and found some new markers with potential clinical significance in patients with gastric cancer. In this paper, we reviewed the progress in serum tumor markers associated with gastric cancer, focusing on the discoveries from serum proteomics and epigenetics researches.

Serum Protein Tumor Markers of Gastric Cancer

Gastric Cancer-Associated Protein Peaks in Serum Proteomics Analysis

   A common characteristic of serum protein tumor markers is of very low expression level in early stage of cancers, therefore the conventional methods for discovery of serum tumor markers have certain limitations. Proteomics analysis can analyze the complex composition of serum and provide the differences in protein expression in many cancers, such as rectum cancer, pancreatic cancer, bladder cancer, ovarian cancer and prostate cancer.

   Lots of studies on gastric cancer have been reported recently (Deininger et al., 2008; Li et al., 2008; Khoder et al., 2009; Wu et al., 2009; Wu et al., 2009; Zhang et al., 2010), and the results showed some clinical significance in the diagnosis of gastric cancer. Lu et al. (2010) compared the difference of proteomic analysis between gastric cancer (n = 34) and normal control (n = 30) and found five different protein peaks, by which a diagnostic model was developed and showed a sensitivity of 94.3% and a specificity of 93.3% in the diagnosis of gastric cancer. The diagnostic value of this model was validated with a new set of serum samples (31 cases of gastric cancer, 30 normal controls) and provided a sensitivity of 90.3% and a specificity of 80.0%, significantly higher than either CEA, CA19-9 alone or the two combined (10% -50%). In addition, they also found a protein peak with sensitivity significantly higher in the diagnostic of stage I / II gastric cancer than stage III / IV gastric cancer, indicating that it was might valuable in the diagnosis of early gastric cancer. Liu et al. (2010) found six gastric cancer serum peptide peaks with good sensitivity (100%) and specificity (75%) in the diagnosis of gastric cancer, which could distinguish early gastric cancer and advanced gastric cancer to some extent. Umemura et al. (2011) found that a 2209m/z protein peak was significantly higher in gastric cancer serum, and its area under the receiver operating characteristic (ROC) curve (AUC) to diagnose stage I gastric cancer (0.715) was greater than those of CEA (0.593) and CA19-9 (0.527). Wang et al. (2007) analyzed the serum protein expression

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profile in 32 cases of gastric adenocarcinoma, 22 cases of peptic ulcer, 32 cases of gastritis and 30 normal subjects and found that the peaks at 2953, 3267, 5341, 5912, 5927 m/z increased and the peaks at 4059, 4213, 4270, 7160 m/z decreased in the serum of patients with gastric cancer; the peak at 5912 m/z was very high in gastric cancer serum with a sensitivity of 81.25% and a specificity of 56.67% in diagnosis of gastric cancer , significantly higher than the existing markers of gastric cancer, but in the normal controls, peptic ulcer and gastritis patients, it was significantly down-regulated, which could be a potential gastric cancer biomarkers. Liang et al. (2004) performed proteomics analysis in 33 patients with gastric adenocarcinoma and 31 healthy subjects and found three differentially expressed protein peaks in which the peak at 5910 m/z increased in gastric cancer patients and showed good diagnostic performance with a sensitivity of 90.91%, a specificity of 93.55% and a positive predictive value of 93.75%. Liu et al. (2009) detected the relative content of serum proteins in 40 patients with gastric cancer, 20 patients with gastric ulcer and 20 normal controls and found no different protein peak in the normal controls, two different protein peaks, the 5910 and 4095 m/z, in one case of gastric ulcer, and 5 significant protein peaks, the 3300, 5329, 4095, 5 910 and 8691 m/z, in the gastric cancer patients; the model created with peaks at 4095, 5910 and 8691 m/z could effectively diagnose gastric cancer with a sensitivity of 92.5% and a specificity of 97.5%. Wang et al. (2008) also detected serum protein fingerprint maps in 26 patients with gastric cancer and 37 patients with superficial gastritis, and they screened six significant protein peaks, the 8587, 6945, 8243, 3899, 7035 and 9943 m/z, by which a diagnostic model was established and showed a sensitivity of 88.5% and a specificity of 97.3% in the diagnosis of gastric cancer. Xue et al. (2009) also explored the feasibility of serum protein fingerprint map for the early diagnosis of gastric cancer. They established 3 models for early diagnosis of gastric cancer with overall sensitivity 96.3%, specificity 73.1% -84.6%, positive predictive value 78.9%-86.7%, and negative predictive value 94.7%-95.7%, indicating that it is valuable in screening early gastric cancer. In addition, Lu et al. (2006) performed the serum protein mass spectrometry in 34 patients with gastric cancer and 30 healthy subjects and developed a discrimination model with the combination of five protein peaks with a sensitivity of 93.3%, a specificity of 94.1% and an accuracy of 93.75% in training set and a sensitivity of 80.0%, a specificity of 73.5%, an accuracy of 76.6% in validation set.

All the reports above show that proteomics analysis of gastric cancer serum is valuable in finding significant protein peaks for the diagnosis of gastric cancer, and the diagnostic models established with these peaks show good performance in the diagnosis of gastric cancer, especially early gastric cancer, and usually better than the classic serum tumor markers. By further analysis, detailed proteins in these peaks could be identified and their significance in the diagnosis of gastric cancer was evaluated. Transthyretin was identified from the different protein peaks in the sera between gastric cancer and normal control and valuable in the early diagnosis of gastric cancer (Zheng et al., 2010). Recently, Ahn et al. (2012) detected 13 candidate protein markers (some of them identified by serum proteomics analysis) in the sera of gastric cancer patients and achieved a accuracy of 88% in the diagnosis of gastric cancer by combined use of these markers. The findings above suggested that there are indeed serum markers with diagnostic value in gastric cancer, but further studies should be performed to identify detailed proteins and evaluate their diagnostic value.

Amyloid A protein

Serum amyloid A protein (SAA) is an acute phase reactive protein which belongs to the heterogeneous proteins of apolipoprotein family. It is mainly synthesized by the liver and significantly elevated in some tumors (Xie et al., 2010; Mittal et al., 2012). Chan et al. (2007) found that the serum SAA concentration in gastric cancer group was much higher than those in gastric ulcer group and healthy control group. It was associated with stage, metastasis and recurrence of gastric cancer, therefore, the authors considered SAA to be useful in the postoperative follow-up of gastric cancer. Liu et al. (2012) found a protein peak highly expressed in patients with gastric cancer and it was analyzed and identified as SAA1 by high performance liquid chromatography, suggesting that SAA was a potential serum marker of gastric cancer. However, the value of SAA in the early diagnosis of gastric cancer required further to be evaluated.

Regenerating gene IV (Reg IV)

Reg IV is a member of the regenerating gene family located on chromosome 1p12 ~ 13.1. Reg IV gene codes a secreted protein consisted of 158 amino acids. Reg IV protein is mainly expressed in gastric parietal cells and intestinal epithelial neuroendocrine cells and is associated with the proliferation and differentiation of gastrointestinal tract cells. Kobayashi et al. (2010) confirmed the role of Reg IV in gastric cancer. They found that the serum levels of Reg IV was significantly elevated in the patients with early gastric cancer, with a sensitivity of 94.5% in the diagnosis gastric cancer, which was much higher than the CEA, CA19-9 and pepsinogen. Tao et al. (2011) also found that the prognosis of gastric cancer patients with positive serum Reg IV expression was significantly worse than that with the negative expression, and it was superior to CEA, CA19-9 in the diagnosis of early gastric cancer. Reg IV protein may be a valuable serum marker for screening and diagnosing early gastric cancer.

14-3-3 protein

The 14-3-3 protein family associates with many cellular proteins that participate in the regulation of various cellular events including apoptosis, cell cycle, spreading, and migration. The overexpression of 14-3-3β protein can stimulate cell proliferation and migration, thus contribute to the growth of tumor. Rodriguez et al. (2005) found that 14-3-3β protein promoted tumor development by the integrin β1 regulation. Tseng et al. (2011) utilized the proteomics technology to analyze the tissue and serum of patients with gastric cancer and found that

14-3-3β protein was up regulated and highly associated with tumor size, lymph node metastasis and low survival rate. Therefore, the 14-3-3β protein was considered as the gastric cancer markers with potential diagnostic value, which could predict gastric cancer metastasis and deterioration.

Another member of the 14-3-3 protein family, 14-3-3ζ protein, was also closely associated with the histological type and infiltration depth of gastric cancer. Zhang et al. (2010) found that 14-3-3ζ protein was expressed in 79.0% of gastric cancer tissues, but only weakly expressed in scattered cells of normal gastric mucosa; the positive rates of 14-3-3ζ protein expression in papillary and tubular adenocarcinoma were significantly higher than those in mucinous adenocarcinoma and signet ring cell carcinoma, and its positive rates in gastric cancer invaded muscularis and serosa were significantly higher than those in gastric cancer invaded mucosa and submucosa.

Soluble vascular adhesion protein-1(VAP-1)

VAP-1 is an endothelial cell adhesion molecule. It mediates the lymphocyte adhesion to endothelial cells and involves in cell-cell and cell-extracellular matrix adhesion, thereby to promote the tumor cell invasion and metastasis. Yasuda et al. (2011) analyzed the serum VAP-1 levels in 107 patients with gastric cancer and 33 healthy controls, and found that the serum VAP-1 level was significantly elevated in patients with gastric cancer, but it would decrease as the disease progressed. It showed that VAP-1 down-regulation was significantly associated with poor prognosis of gastric cancer.

Gastric cancer antigen MG7-Ag (MG7-Ag)

MG7-Ag is a gastric cancer associated antigen with clinical value and discovered through a monoclonal antibody against gastric cancer. Unlike other traditional gastrointestinal tumor-associated antigens, it is high specific and sensitive for gastric cancer. The positive rates of MG7-Ag were 80% to 94% in cancer tissues and 40% to 60% in sera in the patients with gastric cancer (Ren et al., 2000). The sensitivity of the serum MG7-Ag was 81.4% for the diagnosis of gastric cancer (Ren et al., 2011). In the region of China with high incidence of gastric cancer, the sensitivity and specificity of serum MG7-Ag detection were 77.5% and 95.62% for the diagnosis of gastric cancer (Fang et al., 2010;). Chen et al. (1992) also found that MG7-Ag was an important factor for pre-warning of gastric cancer. It was reported that the combined detection of MG7-Ag and COX-2 in 396 cases of gastric precancerous lesions showed that the risk of progress to cancer in the precancerous lesions with positive expression was 22 times greater than that in the precancerous lesions with negative expression (Hong et al., 2010). Therefore, MG7-Ag is a marker for gastric cancer screening and precancerous lesion progressing.

Pepsinogens (PG)

PG is a polypeptide chain protein composed of 375 amino acids and mainly produced by gastric chief cells and mucus neck cells. PG is divided into PG I and PG II depending on the biochemical and immunological features. PG as a marker of gastric cancer remains controversial. In a study with a middle-aged male cohort of Japanese and following-up for 10 years, the results showed that the sensitivity and specificity of serum PG were low for the diagnosis of gastric cancer (Iino et al., 2012). The study in Chinese also showed a similar conclusion (Cao et al., 2012). Actually, PG is a serum marker of chronic atrophic gastritis, not an ideal indicator for the screening and diagnosis of gastric cancer. Irvani et al. (2010) found that the PG I/PG II ratio provided a sensitivity of 96.1% for the diagnosis of atrophic gastritis, and PG I provided a specificity of up to 94.6% for the diagnosis of atrophic gastritis. A Korean study showed that the optimum cut-off value for PG I/PG II ratio was 4 by which the sensitivity and specificity for the diagnosis of atrophic gastritis were 82.6% and 91.7%, respectively (Colarossi et al., 2011). Recently, a case-control study in Peru showed that serum PG I, PG II and PG I/PG II ratio did not achieve the desired sensitivity and specificity (Chae et al., 2011). Therefore, PG detection, especially PG I/PG II ratio have some clinical value for the screening and diagnosis of atrophic gastritis, but the results are different in various ethnics.

Granulin

Granulin (GRN) is an independent growth factor family discovered in recent years, including A, B, C, D and F. They all come from the hydrolysis of the same progranulin (PGRN). Recent studies show that there is a close association between GRN and tumor progression. Loei et al. (2012) found that GRN was frequently expressed in gastric cancer tissues but not in normal gastric epithelia by immunohistochemistry, and it was also elevated in the serum of patients with gastric cancer, particularly early gastric cancer, indicating that serum GRN can provide diagnostic discriminations for gastric cancer patients.

Dickkopf-1(DKK-1)

DKK-1 is a secreted protein which belongs to DKKS family. It plays an important role in the process of tumor development through antagonizing Wnt signaling. It is highly expressed in a variety of malignant tumors. Gomceli et al. (2012) reported the relationship of gastric cancer with DKK-1 in the Turkish population. They detected serum DKK-1 levels in 60 patients with gastric cancer and 69 healthy controls by ELISA, and the results showed that the serum DKK-1 concentration in gastric cancer patients was significantly higher than controls, and when taking the 25 U/mL as cut-off value, the sensitivity and specificity were 100% for the diagnosis of gastric cancer.

Serum Genetic Tumor Markers of Gastric Cancer

In recent years, it has been discovered that the abnormal genetic expression other than DNA sequence change (epigenetics) showed great significance in the occurrence and development of gastric cancer, including microRNAs, DNA methylation, by the mechanisms of the function loss or gain of cancer related genes, increase

of genomic instability, loss of imprinting, etc.. These alterations in genetics could be as biomarkers of gastric cancer for potential clinical applications.

MicroRNA

A microRNA (miRNA, miR) is a small non-coding RNA molecule about 22 nucleotide length, which functions in transcriptional and post-transcriptional regulation of gene expression. A number of studies have confirmed that miRNAs play a critical negative regulatory role in the process of tumor development. The miRNAs closely associated with gastric cancer include miR-21, miR-625, miR-1, miR-20a, miR-378, etc. Wang et al. (2012) found that miR-21 was highly expressed in gastric cancer serum, with significantly higher sensitivity and specificity than the current tumor markers in the diagnosis of gastric cancer. Zheng et al. (2011) found that miR-21 was a marker for detection of tumor cells in peripheral blood, which was highly expressed in 53 cases of preoperative patients with gastric cancer and associated with tumor metastasis, size, TNM staging and histological differentiation. Liu et al. (2011) detected miRNA profiles in 164 cases of gastric cancer serum and found five highly expressed miRNAs (miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p), and the AUC of the five-serum miRNA signature was 0.879 for the diagnosis of gastric cancer, markedly higher than those of the biomarkers carcinoembryonic antigen (CEA) (0.503) and carbohydrate antigen 19-9 (CA19-9) (0.600); they were also associated with the prognosis of gastric cancer. Another study showed that miR-187(*), miR-371-5p and miR-378 were significantly highly expressed in gastric cancer serum, and miR-378 alone could yield a AUC of 0.861, a sensitivity of 87.5% and a specificity of 70.73% in discriminating gastric cancer patients from healthy controls (Liu et al., 2012). Song et al. (2012) investigated serum miRNA expression in the regions of China with high incidence of gastric cancer and found that miR-221, miR-376c and miR-744 were valuable in the diagnosis of early gastric cancer with a sensitivity of 82.4%, a specificity of 58.8% and an accuracy of 79.3%. Other highly expressed miRNAs, such as miR-625, miR-93, miR-16, miR-17-5p, miR-20a, miR-196a, were also found in gastric cancer serum and associated with the recurrence, lymph node metastasis and prognosis of gastric cancer (Song et al., 2012; Tsai et al., 2012; Wang et al., 2012; Wang et al., 2012).

Gene promoter hypermethylation

Gene promoter hypermethylation is one of the mechanisms of gene silencing and is associated with the development of gastric cancer. Zou et al. (2009) analyzed the abnormal methylation of five genes (P16, RUNX3, MGMT, DAPK and RASSF1A) in 20 normal gastric mucosa, 14 intestinal metaplasia, 27 atypical hyperplasia and 16 early gastric adenocarcinoma, and found that the five genes were not abnormally methylated in normal gastric mucosa, but their methylation rates increased gradually from intestinal metaplasia (28.6%), atypical hyperplasia (77.8%) to early gastric adenocarcinoma (87.5%). In the study with serum as samples, there are also similar findings. Chen et al. (2012) detected the promoter region hypermethylation of candidate genes in the serum samples from gastric cancer (GC, n=58), gastric precancerous lesions (GPL, n=46) and normal controls (NC, n=30), and found that the methylation rates of three genes (CHRM2, FAM5C and MYLK) increased with progression from NC to GPL, then to GC, and the hypermethylations of FAM5C and MYLK decreased obviously from preoperative to postoperative evaluation. Additionally, the aberrant methylation of gene promoters is associated with the multiple gastric cancer. Fukuda et al. (2007) found that the hypermethylation of hMLH1 gene was more frequently detected in early multiple gastric cancers than in early solitary gastric cancers, indicating that inactivation of hMLH1 through promoter hypermethylation may be involved in the development of multiple gastric cancer.

The gene promoter region hypermethylation has been considered as a potential test for the diagnosis of gastric cancer. Chen et al. (2012) performed a combined detection of serum FAM5C and MYLK hypermethylation and showed a sensitivity of 77.6% and a specificity of 90% in the diagnosis of gastric cancer, and it was also correlated with tumor size, tumor invasion depth and tumor-node-metastasis (TNM) stage. Lee et al. (2002) detected the promoter methylation of DAP-kinase, E-cadherin, GSTP1, p15 and p16 in the patients with gastric cancer and found the positive rates were 70.3, 75.9, 18.5, 68.5, and 66.7% in primary tumor tissues and 48.1, 57.4, 14.8, 55.6, and 51.9% in serum samples, respectively, and none of the control serum showed aberrant methylation. The hypermethylation as biomarkers for the diagnosis of gastric cancer could be more valuable than the current tumor biomarker (Koike et al., 2005).

Runt-related transcription factor 3 (RUNX3)

RUNX3 is a newly discovered tumor suppressor gene which is located in the short arm 1 of human chromosome l p36.1, and it is the downstream transcription regulatory factor in the TGF-B signaling pathway. Down regulation or silence of RUNX3 can inhibit the apoptosis and induce malignant transformation of gastric mucosal cells. A number of studies have showed that RUNX3 promoter region methylation affects its expression and plays an important role in the occurrence of early gastric cancer. Lu et al. (2012) analyzed the methylation status of RUNX3 promoters in 220 samples of chronic atrophic gastritis, 196 samples of intestinal metaplasia, 134 samples of gastric adenoma, 102 samples of dysplasia, and 202 samples of gastric cancer with paired noncancerous mucosa tissues and corresponding blood specimens, and found that RUNX3 promoter methylation increased with progression of gastric mucosal lesions: the methylation rates were 15.9% in chronic atrophic gastritis, 36.7% in intestinal metaplasia, 41.8% in gastric adenoma, 54.9% in dysplasia and 75.2% in gastric cancer, indicating that circulating RUNX3 methylation was a valuable biomarker for the detection of early gastric cancer. Zheng et al. (2011) found that the combined detection of RUNX3 gene methylation and serum CEA, CA19-9 showed higher sensitivity than CEA and CA19-9 combination in the diagnosis of gastric cancer, but no reduced specificity

Conclusions

The diagnostic performance of classic serum tumor markers, such as CEA, CA19-9, is poor in the diagnosis and screening of gastric cancer, thus lots of studies have been performed to find new biomarkers with potential clinical value. The serum proteomics analysis is frequently applied in the discovery of serum biomarkers of gastric cancer, by which a lot of different protein/peptide peaks have been found in the serum of gastric cancer patients and showed potential diagnostic value, but more serum protein biomarkers of gastric cancer should be identified from these peaks and followed by further confirmation of their significance in clinical practice. The genetic biomarkers of gastric cancer have been another important filed to be investigated, in which the serum microRNAs and gene promoter region hypermethylation have potential application in the diagnosis of gastric cancer. With the development of biological technology, some markers will bring hope to the early screening and diagnosis of gastric cancer.

References

Ahn HS, Shin YS, Park PJ, et al (2012). Serum biomarker panels for the diagnosis of gastric adenocarcinoma. *Br J Cancer*, **106**, 733-9.

Cao XY, Jia ZF, Jin MS, et al (2012). Serum pepsinogen II is a better diagnostic marker in gastric cancer. *World J Gastroenterol*, **18**, 7357-61.

Chae H, Lee JH, Lim J (2011). Clinical utility of serum pepsinogen levels as a screening test of atrophic gastritis. *Korean J Lab Med*, **28**, 201-6.

Chan DC, Chen CJ, Chu HC, et al (2007). Evaluation of serum amyloid A as a biomarker for gastric cancer. *Ann Surg Oncol*, **14**, 84-93.

Chen SZ (1992). The expression of MG7 corresponding antigen in gastrointestinal polyps and its relation with cancer. *Zhonghua Wai Ke Za Zhi*, **30**, 716-8, 777-8.

Colarossi A, Inga R, Prochazka R, et al (2011). Pepsinogen and gastrin in the noninvasive diagnosis of gastric atrophy. A case-control study in Peruvian population. *Rev Gastroenterol Peru*, **31**, 110-5.

Dilege E, Mihmanli M, Demir U, et al (2010). Prognostic value of preoperative CEA and CA 19-9 levels in resectable gastric cancer. *Hepatogastroenterology*, **57**, 674-7.

Duraker N, Celik AN (2001). The prognostic significance of preoperative serum CA 19-9 in patients with resectable gastric carcinoma, comparison with CEA. *J Surg Oncol*, **76**, 266-71.

Deininger SO, Ebert MP, Fü¨tterer A, et al (2008) MALDI imaging combined with hierarchical clustering as a new tool for the interpretation of complex human cancers. *J Proteome Res*, **7**, 5230-6.

Emoto S, Ishigami H, Yamashita H, et al (2012). Clinical significance of CA125 and CA72-4 in gastric cancer with peritoneal dissemination. *Gastric Cancer*, **15**, 154-61.

Fang X, Tie J, Xie Y, et al (2010). Detection of gastric carcinoma-associated antigen MG7-Ag in human sera using surface plasmon resonance sensor. *Cancer Epidemiol*, **34**, 648-51.

Fukuda M, Yokozaki H, Shiba M, et al (2007) Genetic and epigenetic markers to identify high risk patients for multiple early gastric cancers after treatment with endoscopic mucosal resection. *J Clin Biochem Nutr*, **40**, 203-9.

Gomceli I, Bostanci EB, Ozer I, et al (2012). A novel screening biomarker in gastric cancer, serum dickkopf-1. *Hepatogastroenterology*, **59**, 1661-4.

Hong L, Li S, Liu L, et al (2010) The value of MG7-Ag and COX-2 for predicting malignancy in gastric precancerous lesions. *Cell Biol Int*, **34**, 873-6.

Iino C, Shimoyama T, Sasaki Y (2012). Influence of endoscopic submucosal dissection on serum levels of pepsinogens in patients with early gastric cancer. *Dig Endosc*, **24**, 339-42.

Irvani S, Hashemi MR, Moghadam KG, et al (2010). Accuracy of serum pepsinogens I and II, gastrin-17 and anti-helicobacter pylori antibodies in histological diagnoses of atrophic gastritis. *Minerva Gastroenterol Dietol*, **56**, 13-7.

Karpinski P, Myszka A, Ramsey D, et al (2011). Detection of viral DNA sequences in sporadic colorectal cancers in relation to CpG island methylation and methylator phenotype. *Tumour Biol*, **32**, 653-9.

Khoder G, Yamaoka Y, Fauche`re JL, et al (2009). Proteomic Helicobacter pylori biomarkers discriminating between duodenal ulcer and gastric cancer. *J Chromatogr B Analyt Technol Biomed Life Sci*, **877**, 1193-9.

Kobayashi Y, Niwa Y, Tajika M, et al (2010). Serum tumor antigen REG4 as a useful diagnostic biomarker in gastric cancer. *Hepatogastroenterology*, **57**, 1631-4.

Liang Y, Pan CQ (2004). The chemical serum protein fingerprint model application in the diagnosis of gastric adenocarcinoma. *Lab Med*, **27**, 122-6

Liu C, Pan C, Shen J, et al (2012). Identification of serum amyloid A in the serum of gastric cancer patients by protein expression profiling. *Oncol Lett*, **3**, 1259-62.

Liu C, Xu L, Han HB, et al (2009). Preliminary study of serum protein fingerprint screening gastric cancer serum markers. *Bases Clin Med*, **16**, 237-40

Liu H, Zhu L, Liu B, et al (2012). Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. *Cancer Lett*, **316**, 196-203.

Liu R, Zhang C, Hu Z, et al (2011). A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur J Cancer*, **47**, 784-91.

Liu W, Gao X, Cai Q, et al (2010). Identification of novel serum biomarkers for gastric cancer by magnetic bead. *Front Biosci (Elite Ed)*, **2**, 961-71.

Li W, Li JF, Qu Y, et al (2008). Comparative proteomics analysis of human gastric cancer. *World J Gastroenterol*, **14**, 5657-64.

Li Y, Yang Y, Lu M, et al (2011). Predictive value of serum CEA, CA19-9 and CA72.4 in early diagnosis of recurrence after radical resection of gastric cancer. *Hepatogastroenterology*, **58**, 2166-70.

Loei H, Tan HT, Lim TK, et al (2012). Mining the gastric cancer secretome, identification of GRN as a potential diagnostic marker for early gastric cancer. *J Proteome Res*, **11**, 1759-72.

Lu HB, Zhao CH, MaYY, et al (2006). The early gastric clinical significance of serum protein fingerprint diagnostic. *Progress Mod Biomed*, **11**, 120-6

Lu HB, Zhou JH, Ma YY, et al (2010). Five serum proteins identified using SELDI-TOF-MS as potential biomarkers of gastric cancer. *Jpn J Clin Oncol*, **40**, 336-42.

Lu XX, Yu JL, Ying LS, et al (2012). Stepwise cumulation of RUNX3 methylation mediated by Helicobacter pylori infection contributes to gastric carcinoma progression. *Cancer*, **118**, 5507-17.

Mittal A, Poudel B, Pandeya DR, et al (2012). Serum amyloid a as an independent prognostic factor for renal cell carcinoma-a hospital based study from the Western region of Nepal. *Asian Pac J Cancer Prev*, **13**, 2253-5.

Qi Z. Li JF, Qu Y, et al (2007). Gastric cancer and adjacent tissue

tumor-associated gene hypermethylation. *Shanghai Jiaotong Univ Xue Bao (Medical Sciences)*, **27**, 555-8.

Ren J, Chen Z, Juan SJ, et al (2000). Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique. *Cancer*, **88**, 280-5.

Rodriguez LG, Guan JL (2005). 14-3-3 regulation of cell spreading and migration requires a functional amphipathic groove. *J Cell Physiol*, **202**, 285-94.

Song J, Bai Z, Han W, et al (2012). Identification of suitable reference genes for qPCR analysis of serum microRNA in gastric cancer patients. *Dig Dis Sci*, **57**, 897-904.

Song MY, Pan KF, Su HJ, et al (2012). Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. *PLoS One*, **7**, e33608.

Sturgeon CM, Duffy MJ, Hofmann BR, et al (2010). National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem*, **56**, e1-48.

Tao HQ, He XJ, Ma YY, et al (2011). Evaluation of REG4 for early diagnosis and prognosis of gastric cancer. *Hum Pathol*, **42**, 1401-9.

Tsai KW, Liao YL, Wu CW, et al (2012). Aberrant expression of miR-196a in gastric cancers and correlation with recurrence. *Genes Chromosomes Cancer*, **51**, 394-401

Tseng CW, Yang JC, Chen CN, et al (2011). Identification of 14-3-3β in human gastric cancer cells and its potency as a diagnostic and prognostic biomarker. *Proteomics*, **11**, 2423-39.

Umemura H, Togawa A, Sogawa K, et al (2011). Identification of a high molecular weight kininogen fragment as a marker for early gastric cancer by serum proteome analysis. *J Gastroenterol*, **46**, 577-85.

Wang B, Zhang Q (2012). The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol*, **138**, 1659-66.

Wang WJ, Liu CZ, Liu P, et al (2007). SELDI-TOF-MS technology screening gastric cancer serum markers. *Environ Occup Med*, **24**, 143-6.

Wang YS, Shen J, Yu JK, et al (2008). Establish gastric high incidence of gastric cancer serum protein fingerprint diagnostic model. *Zhonghua Yu Fang Yi Xue Za Zhi*, **24**, 677-80

Wang M, Li C, Nie H, et al (2012). Down-regulated miR-625 suppresses invasion and metastasis of gastric cancer by targeting ILK. *FEBS Lett*, **586**, 2382-8.

Wang M, Gu H, Wang S, et al (2012). Circulating miR-17-5p and miR-20a, molecular markers for gastric cancer. *Mol Med Report*, **5**, 1514-20.

Wu C, Luo Z, Chen X, et al (2009). Two-dimensional differential in-gel electrophoresis for identification of gastric cancer-specific protein markers. *Oncol Rep*, **21**, 1429-37.

Wu ZZ, Wang JG, Zhang XL (2009). Diagnostic model of saliva protein finger print analysis of patients with gastric cancer. *World J Gastroenterol*, **15**, 865-70.

Xie X, Ma YT, Yang YN, et al (2010). Polymorphisms in the SAA1/2 gene are associated with carotid intima media thickness in healthy Han Chinese subjects: the Cardiovascular Risk Survey. *PLoS One*, **5**, e13997.

Xue WC, Feng K, Meng DY, et al (2009). Serum protein fingerprint for the early diagnosis of gastric cancer. *J Pract Diagn Ther*, **18**, 153-6.

Yasuda H, Toiyama Y, Ohi M, et al (2011). Serum soluble vascular adhesion protein-1 is a valuable prognostic marker in gastric cancer. *J Surg Oncol*, **103**, 695-9.

Ychou M, Duffour J, Kramar A, et al (2000). Clinical significance and prognostic Value of CA72-4 compared with CEA and CA19-9 in patients with gastric cancer. *Disease Markers*, **16**, 105-10.

Zhang BY, Yuan F, Xiao JC, et al (2010). 14-3-3ζ protein in gastric carcinoma and its significance. *Shanghai Jiaotong Daxue Xue Bao*, **30**, 1129-32.

Zheng ZG, Xu SH, Xu Q, et al (2010). Use of serum protein profiling for early diagnosis of gastric cancer. *Zhonghua Wei Chang Wai Ke Za Zhi*, **13**, 770-3.

Zheng Z, Li M, Zhang G, et al (2010). Identification of human gastric carcinoma biomarkers by differential protein expression analysis using (18)O labeling and NanoLC-MS/MS coupled with laser capture microdissection. *Med Onco*, **27**, 296-303.

Zheng Y, Cui L, Sun W, et al (2011). MicroRNA-21 is a new marker of circulating tumor cells in gastric cancer patients. *Cancer Biomark*, **10**, 71-7.

Zheng Y, Zhang Y, Huang X, et al (2011). Analysis of the RUNX3 gene methylation in serum DNA from esophagus squamous cell carcinoma, gastric and colorectal adenocarcinoma patients. *Hepatogastroenterology*, **58**, 2007-11.

Zou XP, Zhang B, Zhang XQ, et al (2009). Promoter hypermethylation of multiple genes in early gastric adenocarcinoma and precancerous lesions. *Hum Pathol*, **40**, 1534-42.