[Frontiers in Bioscience S2, 403-412, January 1, 2010]

Clinical and biological markers in gastric cancer: update and perspectives

Alessandro Cappellani [1], Antonio Zanghi[1] , Maria Di Vita[1] , Ernesto Zanet[2], Pierfrancesco Veroux[3], Bruno Cacopardo[4], Andrea Cavallaro5, Gaetano Piccolo[1], Emanuele Lo Menzo[6,] Paolo Murabito [7] and Massimiliano Berretta[2]

*[1]University of Catania, Department of Surgery, General and Breast Surgery Unit , University Hospital of Catania, Italy,[2]Department of Medical Oncology, National Cancer Institute – IRCCS, Aviano (PN), Italy,[3]University of Catania, Department of Surgery, Transplantation and Advanced Technologies; Vascular Surgery and Organ Transplant Unit , University Hospital of Catania, Italy,[4]University of Catania, Department of Internal Medicine, "Garibaldi-Nesima" Hospital, University of Catania, Catania Italy, [5]University of Catania, Research Fellow in Surgical Pathology, [6]University of Maryland, Division of Laparoscopic and Bariatric Surgery, Baltimore, MD, USA, [7]Department of Anaesthesia and Intensive Care, Catania University Hospital, Catania, Italy***.**

TABLE OF CONTENTS

1. ABSTRACT

Gastric cancer is the second cause of death from cancer worldwide and the only chance to reach better outcomes lays on an early diagnosis. The need for non-invasive, low-cost tests is invoked also in countries in which imaging and endoscopic screening have already showed the ability to improve early diagnosis and overall survival. Genomic medicine could allow a better understanding of regulatory pathways driving the development and growth of gastric cancer and the characterization of specific molecular targets actually stimulate new drug developments.

The knowledge of the role of Helicobacter pylori (HP) in gastric tumor pathogenesis has put new insides in the understanding of this peculiar disease and enriched the field of gastric biomarkers.

2. INTRODUCTION

Gastric cancer is the 4th cancer as incidence worldwide, with an estimated 934,000 new cases per year in 2002 (8.6% of new cancer cases), behind cancers of the lung, breast, and colon and rectum. It is the second most common cause of death from cancer (700,000 deaths annually). The geographical distribution of stomach cancer is characterized by wide international variations: high risk areas include East Asia (China, Japan), Eastern Europe and parts of Central and South America. Notably approximately 42% of cases occur in China alone. Survival for stomach cancer is moderately good only in Japan (52%). There has been a steady decline in the risk of gastric cancer incidence and mortality over several decades in most country. This decline may be related to improvements in preservation and storage of foods or reduced Helicobacter Pylori transmission in childhood[1].

Early gastric cancer (EGC) is defined as an adenocarcinoma restricted to the gastric mucosa or submucosa, irrespective of the presence or absence of lymph node metastases. 5-year survival rate for EGC exceeds 90%, depending on the degree of tumor invasion (mucosa type T1a or submucosa type T1b) and the presence of metastatic lymph nodes. In contrast, when extended to the muscularis propia or serosa the 5-year survival rate is <10% to 20%[2]. Because most cases of gastric cancer are asymptomatic until advanced stage, the diagnosis of early gastric cancer is difficult.

In Japan[3,4], due to mass screening for gastric cancer, organized by the government and conducted since 1960, with photofluorography, along with an increased number of performed esophagogastroduodenoscopy, outside the mass screening program, the proportion of ECG discovered cases is significantly superior to that of Western countries, with a consequent better survival.

This strategy is considered not cost-effective in low-risk countries and subsequently research of valuable, not invasive and not expensive biomarkers, which could help clinicians to screen population at higher risk of developing gastric cancer, have been encouraged.

Moreover genomic medicine, by exploring tumor molecular profiles, could allow a better understanding of regulatory pathways driving neoplastic development and growth and the behavior of individual gastric cancer.

To simplify the role of gastric cancer markers, they have been divided in: a) screening biomarkers b) advanced disease biomarkers. Several biomarkers have been also evaluated as regards prognosis. This peculiar aspect will be outlined for each singular marker, when demonstrated, in the following presentation.

3. MARKERS RELATED TO SCREENING AND CARCINOGENESIS

The process of gastric cancerogenesis is the consequence of the interaction between host factors, primarily genetic factor, inherited or acquired, and the environment, with alimentary carcinogens, tobacco smoking, and Helicobacter Pylori (HP) infection as the strongest risk factors. Some important discoveries have been done towards identification of particular involved genes involved in the pathogenesis of gastric cancer which can help disease early detection and new insides on the interaction between HP and the host.

3.1. HP

In 1994 HP has been classified as a class I human carcinogen by World Health Organization and the International Agency for Research on Cancer consensus group. Eslick *et al.*[5] meta-analysis demonstrated that HP infection carries around a 2-fold increased risk for the development of gastric cancer. This association is strongest for non-cardia cancer, but holds for both intestinal and diffuse histological types. Since the majority of individuals infected by HP do not develop gastric cancer, additional factors have been proposed to determine which individuals will go on to develop malignancy. Potential factors are bacterial virulence and the role of host genetics. The eradication of the infection before developing of atrophic gastritis is one of the possible way to prevent gastric cancer.

3.1.1. HP virulence factors

Cytotoxin-associated gene A protein (CagA protein). Virulent HP harbour the cag pathogenicity island (cagPAI), a 40 Kb stretch of DNA, which encodes CagA and components of a sophisticated type IV secretion system (T4SS). The T4SS forms a pilus for the injection of virulence factors, such as CagA, into host target cells. CagPAI is present in some but not all HP strains. From several epidemiologic studies, it is now clear that persons with serologic evidence of carrying cag positive strains are at enhanced risk of developing both peptic ulcer disease and non-cardia gastric adenocarcinoma. Most, but not all, CagA proteins contain one or more tyrosine phosphorylation motifs (TPM), which when injected into host epithelial cells, are phosphorylated by Src-like kinases. Phospho-CagA proteins then interact with host molecules involved in a variety of signal transduction pathways affecting host cell gene expression, cytokine release, cell cycle, cell structure. The variety in the number of TPM may in part explain the difference in virulence among HP strains. Also phosphorylation-independent signalling activities of CagA and T4SS have been identified *in vivo* and *in vitro*[6].

Vacuolating cytotoxin A (VacA). The gene encoding VacA is present in virtually all of the HP strains. According to a current model, VacA binds to plasma cell membrane, is internalized by cells and forms anion-

selective membrane channels, inducing the formation of vacuoles, arising due to the swelling of the endosomal compartments. Tegtmeyer *et al.*[7] demonstrated, in HP strains expressing highly active VacA, a protective effect of VacA in host cells obtained through an inhibition of CagA induced responses. In particular VacA seems not to direct influence CagA signal transduction pathways but to interact with epidermal growth factor receptor (EGFR) and HER2/Neu inactivating their activities and subsequently inhibiting host cell elongation and scattering.

TNF-α inducing protein (Tipα). Recently investigated by Suganuma *et al.*[8] is a proposed new HP carcinogenic factor. Tipα is secreted by HP, binds to specific binding molecules, forms a dimer and then penetrate to the nucleus where induces expression of TNFα and chemokine genes. Notably Tipα is secreted in significantly higher amounts in patients with HP associated cancer than HP associated chronic gastritis.

3.2. Host factors

Individual differences in the host response to HP infection, determined by host genetic polymorphisms, might, in part explain why some individuals are more likely to develop gastric cancer than gastritis. Although HP infection play such a central role in initiating the progressive phenotypic modifications in gastric mucosa, the different genetic pattern of the host is equally or more important[9].

Interleukin-1 beta (IL-1β). IL-1β is a pro-inflammatory cytokine and also a potent inhibitor of gastric acid secretion. The IL-1 gene polymorphism was considered as a possible candidate to influence gastric cancer risk. However a meta-analysis[10] and data reported by Shin *et al.*[11] failed to find an overall association between IL-1 gene polymorphism and gastric cancer, even if some studies suggested an association.

Influence of cytokines gene polymorphism on risk of gastric cancer have been studied[9] for Tumor necrosis factor–α(TNF-α), another pro-inflammatory cytokine and Interleukin-10 (IL-10), an anti-inflammatory cytokine that suppresses expression of pro-inflammatory cytokines including IL-1β, TNF-α and interferon-□with contrasting results

3.3. Pepsinogen (PG)

Stomach carcinogenesis is believed to begin with chronic active inflammation of the stomach mucosa, proceeding to extensive atrophy together with intestinal metaplasia, then to displasya and finally to cancer. There is a general agreement that serum PG levels reflect the morphological and functional status of the stomach mucosa. Miki *et al*[12]. demonstrated a correlation between reduction in the area of fundic gland mucosa with chronic atrophic gastritis (CAG) and a reduction in the serum PG I level and PG I/II ratio, proposing PG levels as a marker for CAG progression. Yanaoka *et al.*[13] showed a correlation between an increase in risk of gastric cancer and a reduction in the serum PG I level or the PG I/II ratio, especially in intestinal-type cancer. Conversely, in diffuse-type cancer an increase of PG II levels along with high serum HP antibody correlates with a higher risk of gastric cancer, suggesting the hypothesis that chronic active inflammation directly induces diffuse-type cancer without passing through atrophic gastritis with intestinal metaplasia. The authors concluded that the measurement of serum PG along with HP antibody levels may predict the risk of gastric cancer in each individual with HP related gastritis, in a simple, reproducible, cost-effective way that can be used to screen a large population.

3.4. E-cadherin gene (CDH-1)

CDH-1 gene is localized on chromosome 16q22.1 and encodes a protein that comprises 5 extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. It functions as a calcium-dependent cell-cell adhesion glycoprotein that connects to the actin cytoskeleton through a complex with α-, β- and □-catenin[14]. Loss of protein function through inactivating mutations or promoter methylation leads to development and progression of cancer by lack of inhibition of cell adhesion. Approximately 10% of patients with gastric cancer show familial clustering suggestive of a genetic predisposition and 3% show autosomal dominance and high penetrance. Hereditary diffuse gastric cancer (HDGC)[15] is an autosomal-dominant, inherited cancer syndrome in which inactivating mutations in the CDH-1 gene have been identified in 30% to 50% of patients. CDH-1 mutation carriers have an approximately 70% lifetime risk of developing DGC. Evaluation of CDH-1 mutations allows these patients to undergo to a curative gastrectomy at an early stage of DGC.

3.5. p16

It's an inhibitor of the cyclin D-dependent protein kinase 4/6 and is a cell cycle regulator involved in the inhibition of G1 phase progression. Loss of function of p16 results in higher cyclin D-dependent protein kinase activity and thus leads to aberrant phosphorylation of retinoblastoma, which accelerates cell growth. Inactivation of p16 may occur by deletion or gene mutation but aberrant methylation of CpG island of the promoter region, which ultimately silences transcription of the gene, is the major mechanism of inactivation in gastric cancer. Sun *et al.*[16] proposed detection of aberrant p16 methylation as a useful marker of progression to gastric cancer, in premalignant lesions such as gastric dysplasia.

3.6. Microsatellite instability (MSI)

Microsatellite are DNA regions containing short tandem repeats of 1-6 nucleotide motifs. MSI is a condition characterized by very frequent mutations in microsatellites reflecting a loss of DNA mismatch-repair function. Mismatch repair is a enzyme-mediated mechanism for correcting mispaired nucleotides from DNA. Mispairing often occurs during DNA replication and a specific repair mechanism is required to prevent excessive mutation accumulation. MSI have been used as a molecular marker of a particular kind of tumors: the mismatch-repair-deficient tumors. Interestingly, the association between gastric cancer expressing MSI and clinical characteristics of cancer has become evident over time. Some authors[17]

noted that these tumors are almost always of the intestinal type, are much more frequently located in the distal (antrum) rather than proximal regions (body and cardia) of the stomach, tend to be large in dimension and often do not give rise to lymph node metastases. Ultimately the presence of MSI in gastric cancer identifies a subset of tumors with improved prognosis. On the contrary, Seo *et al.*[18] confirmed that gastric cancers with high MSI have specific clinicopathologic characteristics, such as older age at diagnosis, distal tumor location, increased tumor size and intestinal histologic type but failed to demonstrate a lower incidence of lymph node metastases, lower pTNM stage or better survival rate. Leung *et al.*[19] suggested the hypothesis that a progressive accumulation of MSI in preneoplastic lesions, as intestinal metaplasia, may ultimately lead to gastric cancer development and, consequently, proposed the detection of MSI in preneoplastic lesions as a marker of gastric cancer development.

3.7. hMLH1 or hMSH2 genes

Two components of the DNA mismatch repair genes. Loss of mismatch-repair can either occur as a result of mutations in one of the mismatch-repair genes, most commonly hMLH1 or hMSH2, or alternatively by inactivation of hMLH1 by epigenetic promoter methylation. In gastric cancer, methylation is the cause of MSI in the majority of cases. Fleisher *et al.*[20] demonstrated a significant association of hMLH1 promoter hypermethylation and MSI and a diminished expression of hMLH1 in early gastric neoplasms. Thus hypermethylation-associated inactivation of hMLH1 may be used as a useful marker of early gastric carcinogenesis.

3.8. Reprimo

Reprimo is a downstream mediator of p53-induced G2 cell cycle arrest. When overexpressed, Reprimo induces cell cycle arrest at the G2 phase, suggesting that has tumor suppression function. Functional abrogation of p53 tumor suppressor gene and its downstream mediators is central to the development of human cancers. Epigenetics and in particular alterations of regulatory sequences outside of genes is an emerging field of study in the pathogenesis of cancer. Bernal *et al.*[21] defined a comprehensive methylation profiling in gastric cancer. They identified specific genes associated with signet-ring cell type gastric cancer and showed that only Reprimo has a high frequency of methylation both among gastric cancer primary tissues and plasma samples but rarely in nonmalignant tissue controls. Thus authors propose Reprimo as a potential biomarker for early detection of gastric cancer.

4. MARKERS RELATED TO ADVANCED GASTRIC CANCER

4.1. Classic biomarkers

Due to their low sensitivity and specificity in detecting early primary tumors, classic biomarkers have shown little benefit as a method for screening in the general population. However these markers may be used clinically for the monitoring of tumor recurrence or may be used as prognostic factors because higher levels have been normally observed in advanced disease. Introduction of new techniques as polymerase chain reaction (PCR) may increase the sensibility of detection of these markers respect common immunoassays.

4.1.1. CEA

Chung *et al.*[22] reported higher CEA serum levels in advanced gastric cancer of intestinal-type. Kodama *et al.*[23] confirmed a low positive rate of CEA serum levels in early gastric cancers, similarly to Ca19.9 and Ca72.4. Ohtsuka *et al.*[24] reported false positive elevated CEA levels after gastrectomy. Ucar *et al.*[25] demonstrated a correlation between CEA positivity and presence of liver metastases. Nakanishi *et al.*[26] demonstrated a higher frequency of peritoneal metastases in patients with positive real time-PCR analysis for CEA transcripts in peritoneal washes of gastric cancer patients.

4.1.2. CA 19-9

Kodama *et al.*[23] showed a low positive rate for Ca19.9 in early gastric cancer. Ohtsuka *et al.*[24] observed a false positive increase of Ca 19.9 after gastrectomy, concomitantly to CEA. Ucar *et al.*[25] showed a more frequent significant Ca19.9 serum positivity in patients with lymph nodes, peritoneal and serosal involvement.

4.1.3. Ca 72-4

The 72.4 carbohydrate epitope, contained in high-molecular weight mucin-type glycoprotein, called TAG-72, is detected by monoclonal antibodies CC49 and B72-3.

Kodama *et al.*[23] demonstrated a higher positive rate of serum expression for Ca 72.4 respect CEA and Ca19.9 in advanced gastric cancer, but not in early gastric cancer. Moreover a higher positive rate of expression was seen in the presence of peritoneal dissemination and a first elevation prior to other markers in the presence of recurrence. Mattar *et al.*[27] confirmed increased serum positive expression of Ca 72.4 in advanced gastric disease. Ucar *et al.*[25] showed a more frequent significant Ca 72.4 positivity in patients with lymph nodes, peritoneal and liver involvement and described Ca 72.4 as the only independent prognostic factor for survival among other markers such as CEA, Ca19.9, αFP. Fernandes *et al.*[28] showed a significant correlation between high levels of Ca 72.4 in peritoneal washing and lymph nodes metastasis and serosa involvement by gastric cancer and also with more advanced stage of gastric carcinoma. The levels of Ca 72.4 in the blood correlates significantly with only lymph nodes involvement by gastric carcinoma.

4.1.4. Mg7-Ag

Jin *et al.*[29] demonstrated a lower positive expression of Mg7-Ag in precancerous lesions respect advanced gastric cancer and a correlation of marker level expression with both tumor differentiation and pathological stage. Moreover the sensibility of ELISA and immunochemistry was similar for marker detection. Ren *et al.*[30] proposed an immuno-PCR technique to detect with more sensitivity Mg7-Ag and found a higher intensity of DNA band amplification in patients with metastases than in patients without metastases or with early stage tumors.

4.1.5. Ki-67

Ki-67 labeling index is calculated immunohistochemically evaluating the cell growth-related antigen Ki-67, using the monoclonal antibody MIB-1. Normally the nuclear antigen expressed in proliferating but not in quiescent cells. Consequently, the antibody is used in tumor pathology to detect proliferating cells in neoplastic disease. Tsamandas *et al.*[31] demonstrated a correlation between Ki-67 expression and a poorer survival rate in patients with gastric cancer. Chen *et al.*[32] confirmed the negative correlation between Ki-67 over-expression and survival rates and a positive correlation with clinical stage.

4.2. p53 gene

The p53 gene, located on chromosome 17p, is a tumor-suppressor gene that acts by modulating cell proliferation via control of G1 arrest checkpoint of cell cycle. Abnormalities of the p53 gene have been identified in many malignancies, including gastric cancer. The production of p53 is increased in response to cellular insults or DNA damage. Kopp *et al.*[33] described an increased survival in patients with p53 negative tumors and an increased p53 immunoreactivity in more invasive tumors. Wiksten *et al.*[34] and Al-Moundhri *et al.*[35] confirmed p53 over-expression as an independent negative prognostic factor in gastric cancer. Triantafyllou *et al.*[36] showed immunohistochemically a significantly higher p53 protein expression in advanced cancer compared to early gastric cancer.

4.3. p21 gene

p21 is a tumor suppressor gene which encodes proteins that are activated by p53 and induces cell-cycle arrest by inhibition of kinase activity of cyclin/cyclin dependent kinase complexes regulating cell-cycle progression. Several authors have reported that over-expression of p21 in gastric cancer results in improved outcomes and a minor tumor propensity to metastatize[37, 38, 35] although a few studies[34] reported opposite results.

4.4. Bcl-2 gene

Bcl-2 is a gene located at choromosome 18q21. Bcl-2 gene has been implied in various ways in apoptosis, both as a pro-apoptotic or an antiapoptotic factor. Lee *et al.*[39] showed a significant negative correlation of bcl-2 positive expression with depth of invasion and lymph node metastasis. Moreover patients with bcl-2 positive tumors had a rather better survival than those with bcl-2 negative tumors. On the contrary, Kopp *et al.*[33] demonstrated an association between bcl-2 negative tumors and an increased survival and between bcl-2 immunoreactivity and intestinal type gastric cancers.

4.5. Matrix metalloproteinases (MMPs)

The MMPs are a family of zinc-containing proteases, which collectively are capable of degrading all components of the extracellular matrix. There are currently at least 24 human MMPs. Albo *et al.*[40] demonstrated how thrombospondin 1 (TSP-1), an extracellular matrix glycoprotein, up-regulates MMP-9 expression in gastric cancer cells and that MMP-9, TSP-1 intense immunohistochemical staining correlates with high grade, lymph node metastases, microvessel invasion and advanced stage gastric cancer. Dragutinovic *et al.*[41] confirmed correlation between higher serum determination of MMP-9 and advanced stage gastric cancer. Huachuan *et al.*[42] showed a correlation between MMP-7 expression in primary foci of gastric cancer and tumor size, invasive depth, metastasis and TNM staging and an increased angiogenesis. Fujimoto *et al.*[43] described the correlation between a contemporary over-expression of protease-activated receptors (PARs) and MMP-1 and histological stage, depth of wall invasion, lymph node metasteses and peritoneal dissemination. Moreover these patients had a significantly poorer prognosis than those not expressing both PARs and MMP-1.

4.6. Phosphatase and tensin homologue deleted on chromosome ten/mutated in multiple advanced cancers 1 (PTEN/MMAC1 gene)

PTEN/MMAC1 gene is a tumor suppressor gene, located on chromosome band 10q23.3, which encodes a 403-amino acid, dual specificity protein phospatase. Protein tyrosine phosphatase level, is determined between protein tyrosine kinase and protein tyrosine phosphatase activities. The imbalance between the two enzymes affects cell signal transference and cell division, thus leading to malignance of cells. Wang *et al.*[44] demonstrated that mutations of the PTEN/MMAC1 gene do not occur at a significant rate in human advanced gastric carcinoma, but however the rare clustered mutation site (exon 2-6) perhaps suggest a role of the gene in carcinogenesis. Guo *et al.*[45] showed a significant correlation between PTEN protein expression and infiltrating depth, lymph nodes metastasis and pTNM staging and an increased expression in well-and moderately-differentiated gastric cancers.

4.7 Urokinase-type plasminogen activator (u-PA)

Degradation of extracellular matrix (ECM) and basement membrane is essential for tumor invasion and metastasis. The ECM is degraded by extracellular proteolytic enzymes, such as metalloproteases and serine proteases. Plasminogen activators (PA) catalyze the conversion of the inactive proenzyme plasminogen to plamin. Plasmin acts to degrade the ECM and activates latent enzyme, such as type-IV collagenase. Among the plasminogen activators, urokinase-type plasminogen activator (u-PA) and u-PA receptor (u-PAR) have been reported to play an important role in tumor progression. Zhang *et al.*[46] demonstrated an increased expression of u-PA or u-PAR mRNA in those patients with serosal invasion, lymph node metastasis, vessel invasion, advanced stage of diseases, and distant metastasis and a significant lower survival rate for these patients. On the contrary, Luebke *et al.*[47] failed to find a significant correlation between u-PA expression and overall survival in gastric cancer patients and proposed caution in the u-PA system use as a defined prognostic marker. Wu *et al.*[48] found association between u-PA exon 6 polymorphism and invasive gastric cancer but not correlation with survival. Iwamoto *et al.*[49] demonstrated a role of cag A-positive HP strain in the increased levels of u-PA and u-PAR in gastric cancer cells and decreased levels of u-PA and u-PAR with COX-2 inhibitors and ProstaglandinE2 (PGE2) receptor

antagonist, as if COX-2-PGE2 pathway should involved in HP u-PA and u-PAR induction. uPA mRNA and/or uPAR.

4.8. Epidermal growth factor receptor (EGFR)

The epidermal growth factor receptor gene, also called ERBB, is located at chromosomal region 7p12 and encodes a 170-KDa transmembrane tyrosine kinase receptor, which is the member of the EGFR family. The EGFR is activated by binding to its ligands such as epidermal growth factor (EGF) or transforming growth factor-alpha (TGF-α), resulting in homodimerization or heterodimerization with another member of the EGFR family. The receptor activation is followed by phosphorylation of specific tyrosine residues within the cytoplasmic tail, stimulating the downstream signaling pathway that regulates cell proliferation, migration, adhesion, differentiation and survival. Kim *et al.*[50] evaluated EGFR status of gastric carcinoma using both immunohistochemistry and fluorescence *in situ* hybridization. The authors described a significant correlation between EGFR over-expression and older age, moderately or poorly differentiated histology and higher stage disease. Moreover patients with EGFR over-expression had an unfavourable prognosis. Conversely, Matsubara *et al.*[51] failed to find a correlation between EGFR over-expression and overall survival. The same results were obtained for HER2 (also known as erbB-2), an homolog of EGFR, one of the preferred co-receptors for the formation of dimmers with EGFR and a member of the erbB gene family. Baek *et al.*[52] showed how EGF expression, a ligand of EGFR, induces u-PAR expression via ERK-1/2, AP-1 and NF-kappaB signaling pathway and in turn stimulates invasiveness in human gastric cells.

4.9. Vascular endothelial growth factor (VEGF)

Tumor growth requires neoangiogenesis. The VEGF superfamily of endothelial growth factors seems to play a crucial role in the proliferation and migration of endothelial cells, providing nourishment to growing tumors and making the tumor cell establish continuity with the host vasculature. Lieto *et al.*[53] demonstrated a correlation between VEGF over expression and a worse survival. Interestingly, the valuation of VEGF over-expression along with EGFR, seems to better estimate than TNM the risk of cancer-related death and, within the same TNM stage, to individuate high-risk patients. Arigami *et al.*[54] showed a correlation between VEGF-C and –D over-expression in primary tumor obtained by RT-PCR and the presence of lymph node micrometastasis in early gastric cancer. These results were confirmed by Morita *et al.*[55] that found a relation between VEGF-C expression and lymph nodes metastases only in early gastric cancer. Kondo *et al.*[56] demonstrated a correlation between positive immnunoreactivity of both VEGF-C and –A and lymph node metastasis in gastric cancer. Vidal *et al.*[57] reported positive VEGF immunostaining as the only angiogenic marker, among several tested angiogenic factors, with independent prognostic significance for poor clinical outcome.

4.10. Insulin-like growth factor type I receptor (IGF-IR)

The IGF-IR is a heterodimer of α and β chains. Binding of the ligands (IGF-I and IGF-II) to IGF-IR causes receptor autophosphorylation and leads to activation of multiple signaling pathways. Matsubara *et al.*[51] showed a high rate of IGF-IR-positive expression, on immunohistochemical assay, in gastric cancer and a relation between such expression and poor outcomes. Min *et al.*[58] demonstrated an enhanced response to chemo-radiotherapy and an inhibition of gastric cancer growth obtained through blockade of IGF-IR, favoring IGF-IR as a potential target for cancer therapy.

4.11. K-sam-II gene

K-sam gene encodes a member the heparin-binding growth factor receptor tyrosine kinase and has at least four transcriptional variants. One of these, Type II, encodes a receptor for keratinocyte growth factor (KGF). Amplification of the K-sam gene seems to be restricted to advanced diffuse or scirrhous-type gastric carcinomas but not in intestinal-type cancers. Over-expression of this gene in gastric carcinoma is associated with a poorer prognosis. Toyokava *et al.*[59] showed a significantly worse prognosis in patients over-expressing both K-sam and KGF.

4.12. C-Met gene

C-met gene encodes a receptor for hepatocyte growth factor and is amplified both in intestinal and diffuse-type gastric cancer. Drebber *et al.*[60] described a correlation between c-met over-expression and a poor prognosis.

4.13. Phospholipase A2 group IIA (PLA2G2A)

Phospholipase A2 (PLA2) catalyzes hydrolysis of the sn-2 fatty acyl ester bond of phosphoglycerides, releasing fatty acids from membrane stores, such as arachidonic acid and lysophospholipids. At least 15 human genes encode different PLA2 enzymes, distinguished in secreted/extracellular and cytosolic forms. PLA2G2A is a secreted PLA2. Leung *et al.*[61] demonstrated a possible role of regulation of this enzyme in limiting progression of gastric cancer and appearance of metastases. Ganesan *et al.*[62] demonstrated PLA2G2A as a direct target of Wnt/β-catenin signaling in gastric cancer cells which through negative regulation of downstream genes such as *S100A4* and *NEDD9* inhibits gastric cells invasion and metastasis. Moreover PLA2G2A expression is decreased in metastatic tumors and epigenetic silencing through methylation of PLA2G2A promoter may play a role.

4.14. Survivin

Survivin is a member of IAPs family, a group of important apoptosis regulatory proteins. IAPs family proteins are generally over-expressed in many solid tumors including gastric cancer. Many chemotherapeutic agents exert anticancer effects by down-regulating IAPs family members and thus targeting IAP family members can be a promising approach for cancer therapy. Moreover Song *et al.*[63] demonstrated that survivin expression is significantly related to large tumor size and to a lower 5-year survival rate in a group of stage III gastric adenocarcinomas. Yie *et al.*[64] demonstrated a correlation between detection of survivin-expressing circulating cancer cells and prediction of metastasis or recurrence in gastric cancer. Dalal *et al.*[65] attempted to detect micrometastases in peritoneal washing of gastric cancer patients by the reverse trascriptase

polymerase chain reaction (RT-PCR). They lacked to show an additional benefit from the detection of CK20, MUC2 and survivin in addition to CEA, but they identified RT-PCR positive/cytology negative patients that probably represent a high risk population for peritoneal recurrence after curative surgery and death.

4.15. Aurora Kinase A (AURKA)

Aurora kinases (AK)[66] are a family of serine/threonine protein kinases. These kinases play a fundamental role in the control of the cell division process. In particular AK control precise centrosome function which ensures equal segregation of replicated chromosomes into two daughter cells. In experimental models, overexpression of AK can induce chromosomal instability and malignant transformation. Conversely, down regulation of AK expression cause mitotic arrest and apoptosis in tumor cell lines. Three AK family members heve been identified in mammalian cells: A, B and C. Ju *et al.*[67] demonstrated how expression of AUKRA single nucleotide polymorphisms, through a higher kinase activity, is associated with gastric cancer progression. Moreover these family of kinases gained interest as potential drug targets.

4.16. Cyclooxygenase-2 (COX-2)

COX-2 is the inducible key enzyme of arachidonic acid metabolism. Lazar *et al.*[68] demonstrated a predominant immunohistochemical expression of COX-2 in gastric carcinomas of intestinal type and precursory lesions. Moreover they showed that COX-2 expression is significantly correlated with cancer invasion, presence of metastases in the regional lymph nodes, the pTNM stage and intense angiogenesis activity. Walduck *et al.*[69] showed an up-regulated expression of COX-2 gene in the gastric mucosa during Helicobacter Pylori (HP) infection and they identified a subset of COX-2 dependent genes, in HP infection *in vivo*, including those influencing gastric physiology, epithelial barrier functions, inflammation, apoptosis and proliferation.

4.17. MASPIN

Member of the serpin family of protease inhibitors known to have tumor suppressor activity. The frequency of maspin expression in gastric adenocarcinoma seems to be associated with the stage of gastric cancer and lymph node metastasis[70]. Anti-maspin antibodies have been successfully used to improve detection of minimal amounts of gastric cancer cells in peritoneal washing[71].

5. CONCLUSIONS

The poor outcome still now affecting most of gastric cancers has stimulated a reasonably large mass of studies aimed to find new and more reliable tests and biomarkers for screening, for prognosis, for therapeutic decisions and for evaluation of response to treatment.

The discovery of HP role in gastric cancer cancerogenesis have added to both bacterial virulence factors and host factors a role as markers.

Genetic and molecular studies have further improved the knowledge of factors and genes implicated in various steps of initiation, growth and progression of cancer and some of these have been proposed as biomarkers.The role of so called classic biomarkers have been redesigned.

6 ACKNOWLEDGEMENTS

All the authors equally participated in conceiving, drafting and revising the manuscript

7 REFERENCES

1. Parkin D. M., F. Bray, J. Ferlay and P. Pisani: Global cancer statistics, 2002. *CA Cancer J Clin*, 55(2), 74-108 (2005)

2. Nomura S. and M. Kaminishi: Surgical treatment of early gastric cancer. *Dig Surg*, 24(2), 96-100 (2007)

3. Suzuki H., T. Gotoda, M. Sasako and D. Saito: Detection of early gastric cancer: misunderstanding the role of mass screening. *Gastric Cancer*, 9(4), 315-9 (2006)

4. Hamashima C., D. Shibuya, H. Yamazaki, K. Inoue, A. Fukao, H. Saito and T. Sobue: The Japanese guidelines for gastric cancer screening. *Jpn J Clin Oncol*, 38(4), 259-67 (2008)

5. Eslick G. D.: Helicobacter pylori infection causes gastric cancer? A review of the epidemiological, meta-analytic, and experimental evidence. *World J Gastroenterol*, 12(19), 2991-9 (2006)

6. Blaser M. J. : The biology of cag in the helicobacter pylori-human interaction. *Gastroenterology*, 128: 1512-5 (2005)

7. Tegtmeyer N., D. Zabler, D. Schmidt, R. Hartig, S. Brandt and S. Backert: Importance of EGF receptor, HER2/Neu and Erk1/2 kinase signalling for host cell elongation and scattering induced by the Helicobacter pylori CagA protein: antagonistic effects of the vacuolating cytotoxin VacA. *Cell Microbiol*, 11(3), 488-505 (2009)

8. Suganuma M., K. Yamaguchi, Y. Ono, H. Matsumoto, T. Hayashi, T. Ogawa, K. Imai, T. Kuzuhara, A. Nishizono and H. Fujiki: TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells. *Int J Cancer*, 123(1), 117-22 (2008)

9. Lochhead P. and E. M. El-Omar: Gastric cancer. *Br Med Bull*, 85, 87-100 (2008)

10. Kamangar F., C. Cheng, C. C. Abnet and C. S. Rabkin: Interleukin-1B polymorphisms and gastric cancer risk--a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 15(10), 1920-8 (2006)

11. Shin W. G., J. S. Jang, H. S. Kim, S. J. Kim, K. H. Kim, M. K. Jang, J. H. Lee, H. J. Kim and H. Y. Kim: Polymorphisms of interleukin-1 and interleukin-2 genes in patients with gastric cancer in Korea. *J Gastroenterol Hepatol*, 23(10), 1567-73 (2008)

12. Miki K.and Y. Urita: Using serum pepsinogens wisely in a clinical practice. *J Dig Dis*, 8(1), 8-14 (2007)

13. Yanaoka K., M. Oka, C. Mukoubayashi, N. Yoshimura, S. Enomoto, M. Iguchi, H. Magari, H. Utsunomiya, H. Tamai, K. Arii, H. Ohata, M. Fujishiro, T. Takeshita, O. Mohara and M. Ichinose: Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol Biomarkers Prev*, 17(4), 838-45 (2008)

14. Norton J. A., C. M. Ham, J. Van Dam, R. B. Jeffrey, T. A. Longacre, D. G. Huntsman, N. Chun, A. W. Kurian and J. M. Ford: CDH1 truncating mutations in the E-cadherin gene: an indication for total gastrectomy to treat hereditary diffuse gastric cancer. *Ann Surg*, 245(6), 873-9 (2007)

15. Cisco R. M., J. M. Ford and J. A. Norton: Hereditary diffuse gastric cancer: implications of genetic testing for screening and prophylactic surgery. *Cancer,* 113(7 Suppl), 1850-6 (2008)

16. Sun Y., D. Deng, W. C. You, H. Bai, L. Zhang, J. Zhou, L. Shen, J. L. Ma, Y. Q. Xie and J. Y. Li: Methylation of p16 CpG islands associated with malignant transformation of gastric dysplasia in a population-based study. *Clin Cancer Res*, 10(15), 5087-93 (2004)

17. Simpson A. J., O. L. Caballero and S. D. Pena: Microsatellite instability as a tool for the classification of gastric cancer. *Trends Mol Med*, 7(2), 76-80 (2001)

18. Seo H. M., Y. S. Chang, S. H. Joo, Y. W. Kim, Y. K. Park, S. W. Hong and S. H. Lee: Clinicopathologic characteristics and outcomes of gastric cancers with the MSI-H phenotype. *J Surg Oncol*, 99(3), 143-7 (2009) doi:10.1002/jso.21220

19. Leung W. K., J. J. Kim, J. G. Kim, D. Y. Graham and A. R. Sepulveda: Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. *Am J Pathol*, 156(2), 537-43 (2000)

20. Fleisher A. S., M. Esteller, G. Tamura, A. Rashid, O. C. Stine, J. Yin, T. T. Zou, J. M. Abraham, D. Kong, S. Nishizuka, S. P. James, K. T. Wilson, J. G. Herman and S. J. Meltzer: Hypermethylation of the hMLH1 gene promoter is associated with microsatellite instability in early human gastric neoplasia. *Oncogene*, 20(3), 329-35 (2001)

21. Bernal C., F. Aguayo, C. Villarroel, M. Vargas, I. Diaz, F. J. Ossandon, E. Santibanez, M. Palma, E. Aravena, C. Barrientos and A. H. Corvalan: Reprimo as a potential biomarker for early detection in gastric cancer. *Clin Cancer Res*, 14(19), 6264-9 (2008)

22. Chung H. W., J. W. Kim, J. H. Lee, S. Y. Song, J. B. Chung, O. H. Kwon and J. B. Lim: Comparison of the Validity of three Biomarkers for Gastric Cancer Screening: Carcinoembryonic Antigen, Pepsinogens, and High Sensitive C-reactive Protein. J *Clin Gastroenterol* 43(1), 19-26 (2008)

23. Kodama I., K. Koufuji, S. Kawabata, S. Tetsu, Y. Tsuji, J. Takeda and T. Kakegawa: The clinical efficacy of CA 72-4 as serum marker for gastric cancer in comparison with CA19-9 and CEA. *Int Surg*, 80(1), 45-8 (1995)

24. Ohtsuka T., S. Sato, Y. Kitajima, M. Tanaka, Y. Nakafusa and K. Miyazaki: False-positive findings for tumor markers after curative gastrectomy for gastric cancer. *Dig Dis Sci*, 53(1), 73-9 (2008)

25. Ucar E., E. Semerci, H. Ustun, T. Yetim, C. Huzmeli and M. Gullu: Prognostic value of preoperative CEA, CA 19-9, CA 72-4, and AFP levels in gastric cancer. *Adv Ther*, 25(10), 1075-84 (2008)

26. Nakanishi H., Y. Kodera, Y. Yamamura, S. Ito, T. Kato, T. Ezaki and M. Tatematsu: Rapid quantitative detection of carcinoembryonic antigen-expressing free tumor cells in the peritoneal cavity of gastric-cancer patients with real-time RT-PCR on the lightcycler. *Int J Cancer*, 89(5), 411-7 (2000)

27. Mattar R., C. R. Alves de Andrade, G. M. DiFavero, J. J. Gama-Rodrigues and A. A. Laudanna: Preoperative serum levels of CA 72-4, CEA, CA 19-9, and alpha-fetoprotein in patients with gastric cancer. *Rev Hosp Clin Fac Med Sao Paulo*, 57(3), 89-92 (2002)

28. Fernandes L. L., L. C. Martins, C. A. Nagashima, A. C. Nagae, D. R. Waisberg and J. Waisberg: CA72-4 antigen levels in serum and peritoneal washing in gastric cancer. Correlation with morphological aspects of neoplasia. *Arq Gastroenterol*, 44(3), 235-9 (2007)

29. Jin B., X. Wang, Y. Jin, W. Xia, B. Chen, L. Liu, Z. Chen, L. Hong, W. Du, K. Yan, H. Wang, D. Yuan, X. Hui, L. He, F. Zhang, Y. Zhao, K. Wu and D. Fan: Detection of serum gastric cancer-associated MG7-Ag from gastric cancer patients using a sensitive and convenient ELISA method. *Cancer Invest*, 27(2), 227-33 (2009)

30. Ren J., Z. Chen, S. J. Juan, X. Y. Yong, B. R. Pan and D. M. Fan: Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique. Cancer, 88(2), 280-5 (2000)

31. Tsamandas A. C., D. Kardamakis, P. Tsiamalos, A. Liava, V. Tzelepi, V. Vassiliou, T. Petsas, K. Vagenas, V. Zolota and C. D. Scopa: The potential role of Bcl-2 expression, apoptosis and cell proliferation (Ki-67 expression) in cases of gastric carcinoma and correlation with classic prognostic factors and patient outcome. *Anticancer Res*, 29(2), 703-9 (2009)

32. Chen L., X. Li, G. L. Wang, Y. Wang, Y. Y. Zhu and J. Zhu: Clinicopathological significance of overexpression of TSPAN1, Ki67 and CD34 in gastric carcinoma. *Tumori,* 94(4), 531-8 (2008)

33. Kopp R., J. Diebold, I. Dreier, C. Cramer, J. Glas, G. Baretton and K. W. Jauch: Prognostic relevance of p53 and

bcl-2 immunoreactivity for early invasive pT1/pT2 gastric carcinomas: indicators for limited gastric resections? *Surg Endosc*, 19(11), 1507-12 (2005)

34. Wiksten J. P., J. Lundin, S. Nordling, A. Kokkola and C. Haglund: Comparison of the prognostic value of a panel of tissue tumor markers and established clinicopathological factors in patients with gastric cancer. *Anticancer Res*, 28(4C), 2279-87 (2008)

35. Al-Moundhri M. S., V. Nirmala, I. Al-Hadabi, K. Al-Mawaly, I. Burney, M. Al-Nabhani, V. Thomas, S. S. Ganguly and C. Grant: The prognostic significance of p53, p27 kip1, p21 waf1, HER-2/neu, and Ki67 proteins expression in gastric cancer: a clinicopathological and immunohistochemical study of 121 Arab patients. i, 91(4), 243-52 (2005)

36. Triantafyllou K., P. Kitsanta, D. G. Karamanolis, C. Kittas and S. D. Ladas: Epithelial cell turnover, p53 and bcl-2 protein expression during oncogenesis of early and advanced gastric cancer in a Western population. *Dig Liver Dis*, 40(1), 39-45 (2008)

37. Baldus S. E., P. M. Schneider, S. P. Monig, T. K. Zirbes, S. Fromm, W. Meyer, J. Glossmann, S. Schuler, J. Thiele, A. H. Holscher and H. P. Dienes: p21/waf1/cip1 in gastric cancer: associations with histopathological subtypes, lymphonodal metastasis, prognosis and p53 status. *Scand J Gastroenterol*, 36(9), 975-80 (2001)

38. Mattioli E., P. Vogiatzi, A. Sun, G. Abbadessa, G. Angeloni, D. D'Ugo, D. Trani, J. P. Gaughan, F. M. Vecchio, G. Cevenini, R. Persiani, A. Giordano and P. P. Claudio: Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer. *J Cell Physiol*, 210(1), 183-91 (2007)
doi:10.1002/jcp.20833

39. Lee H. K., H. S. Lee, H. K. Yang, W. H. Kim, K. U. Lee, K. J. Choe and J. P. Kim: Prognostic significance of Bcl-2 and p53 expression in gastric cancer. *Int J Colorectal Dis*, 18(6), 518-25 (2003)

40. Albo D., T. Shinohara and G. P. Tuszynski: Up-regulation of matrix metalloproteinase 9 by thrombospondin 1 in gastric cancer. *J Surg Res*, 108(1), 51-60 (2002)

41. Dragutinovic V., L. Izrael-Zivkovic and N. Radovanovic: Relation of matrix metalloproteinase-9 to different stages of tumors in the serum of gastric cancer. *Dig Dis Sci*, 54(6), 1203-7 (2009)

42. Huachuan Z., L. Xiaohan, S. Jinmin, C. Qian, X. Yan and Z. Yinchang: Expression of matrix metalloproteinase-7 involving in growth, invasion, metastasis and angiogenesis of gastric cancer. *Chin Med Sci* J, 18(2), 80-6 (2003)

43. Fujimoto D., Y. Hirono, T. Goi, K. Katayama and A. Yamaguchi: Prognostic value of protease-activated receptor-1 (PAR-1) and matrix metalloproteinase-1 (MMP-1) in gastric cancer. Anticancer Res, 28(2A), 847-54 (2008)

44. Wang J. Y., T. J. Huang, F. M. Chen, M. C. Hsieh, S. R. Lin, M. F. Hou and J. S. Hsieh: Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in advanced gastric carcinomas. *Virchows Arch*, 442(5), 437-43 (2003)

45. Guo C. Y., X. F. Xu, J. Y. Wu and S. F. Liu: PCR-SSCP-DNA sequencing method in detecting PTEN gene mutation and its significance in human gastric cancer. World J Gastroenterol, 14(24), 3804-11 (2008)

46. Zhang F. H., Y. N. Zhou, G. Z. Li, Q. Li, B. Lu, Z. Y. Zhang, Z. Q. Wu, P. Fan and T. J. Hao: [Expression of cyclooxygenase-2 and urokinase plasminogen activator in gastric carcinoma and the clinical significance thereof]. Zhonghua Yi Xue Za Zhi, 88(46), 3279-82 (2008)

47. Luebke T., S. E. Baldus, D. Spieker, G. Grass, E. Bollschweiler, P. M. Schneider, J. Thiele, H. P. Dienes, A. H. Hoelscher and S. P. Moenig: Is the urokinase-type plasminogen activator system a reliable prognostic factor in gastric cancer? Int J Biol Markers, 21(3), 162-9 (2006)

48. Wu C. Y., M. S. Wu, Y. J. Chen, H. P. Chen, C. J. Chen, C. S. Chang and J. T. Lin: Clinicopathological significance of urokinase-type plasminogen activator genotypes in gastric cancer. Hepatogastroenterology, 55(86-87), 1890-4 (2008)

49. Iwamoto J., Y. Mizokami, K. Takahashi, T. Matsuoka and Y. Matsuzaki: The effects of cyclooxygenase2-prostaglandinE2 pathway on Helicobacter pylori-induced urokinase-type plasminogen activator system in the gastric cancer cells. Helicobacter, 13(3), 174-82 (2008)

50. Kim M. A., H. S. Lee, H. E. Lee, Y. K. Jeon, H. K. Yang and W. H. Kim: EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. Histopathology, 52(6), 738-46 (2008)

51. Matsubara J., Y. Yamada, Y. Hirashima, D. Takahari, N. T. Okita, K. Kato, T. Hamaguchi, K. Shirao, Y. Shimada and T. Shimoda: Impact of insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and HER2 expressions on outcomes of patients with gastric cancer. Clin Cancer Res, 14(10), 3022-9 (2008)

52. Baek M. K., M. H. Kim, H. J. Jang, J. S. Park, I. J. Chung, B. A. Shin, B. W. Ahn and Y. D. Jung: EGF stimulates uPAR expression and cell invasiveness through ERK, AP-1, and NF-kappaB signaling in human gastric carcinoma cells. *Oncol Rep*, 20(6), 1569-75 (2008)

53. Lieto E., F. Ferraraccio, M. Orditura, P. Castellano, A. L. Mura, M. Pinto, A. Zamboli, F. De Vita and G. Galizia: Expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) is an independent prognostic indicator of worse outcome in gastric cancer patients. *Ann Surg Oncol*, 15(1), 69-79 (2008)

54. Arigami T., S. Natsugoe, Y. Uenosono, S. Yanagita, K. Ehi, H. Arima, Y. Mataki, A. Nakajo, S. Ishigami and T. Aikou: Vascular endothelial growth factor-C and -D expression correlates with lymph node micrometastasis in pN0 early gastric cancer. *J Surg Oncol*, 99(3), 148-53 (2009)

55. Morita H., Y. Ishikawa, Y. Akishima-Fukasawa, K. Ito, Y. Akasaka, C. Nishimura, Y. Igarashi, K. Miki and T. Ishii: Histopathological predictor for regional lymph node metastasis in gastric cancer. *Virchows Arch*, 454(2), 143-51 (2009)

56. Kondo K., T. Kaneko, M. Baba and H. Konno: VEGF-C and VEGF-A synergistically enhance lymph node metastasis of gastric cancer. *Biol Pharm Bull*, 30(4), 633-7 (2007)

57. Vidal O., A. Soriano-Izquierdo, M. Pera, J. I. Elizalde, A. Palacin, A. Castells, J. M. Pique, A. Volant and J. P. Metges: Positive VEGF immunostaining independently predicts poor prognosis in curatively resected gastric cancer patients: results of a study assessing a panel of angiogenic markers. *J Gastrointest Surg,* 12 (6), 1005-14 (2008)

58. Min Y., Y. Adachi, H. Yamamoto, A. Imsumran, Y. Arimura, T. Endo, Y. Hinoda, C. T. Lee, S. Nadaf, D. P. Carbone and K. Imai: Insulin-like growth factor I receptor blockade enhances chemotherapy and radiation responses and inhibits tumour growth in human gastric cancer xenografts. *Gut,* 54(5), 591-600 (2005)

59. Toyokawa T., M. Yashiro and K. Hirakawa: Co-expression of keratinocyte growth factor and K-sam is an independent prognostic factor in gastric carcinoma. *Oncol Rep*, 21(4), 875-80 (2009)

60. Drebber U., S. E. Baldus, B. Nolden, G. Grass, E. Bollschweiler, H. P. Dienes, A. H. Holscher and S. P. Monig: The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. *Oncol Rep*, 19(6), 1477-83 (2008)

61. Leung S. Y., X. Chen, K. M. Chu, S. T. Yuen, J. Mathy, J. Ji, A. S. Chan, R. Li, S. Law, O. G. Troyanskaya, I. P. Tu, J. Wong, S. So, D. Botstein and P. O. Brown: Phospholipase A2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. *Proc Natl Acad Sci U S A*, 99(25), 16203-8 (2002)

62. Ganesan K., T. Ivanova, Y. Wu, V. Rajasegaran, J. Wu, M. H. Lee, K. Yu, S. Y. Rha, H. C. Chung, B. Ylstra, G. Meijer, K. O. Lian, H. Grabsch and P. Tan: Inhibition of gastric cancer invasion and metastasis by PLA2G2A, a novel beta-catenin/TCF target gene. *Cancer Res,* 68(11), 4277-86 (2008)

63. Song K. Y., C. K. Jung, W. S. Park and C. H. Park: Expression of the antiapoptosis gene Survivin predicts poor prognosis of stage III gastric adenocarcinoma. Jpn J Clin Oncol, 39(5), 290-6 (2009)

64. Yie S. M., B. Lou, S. R. Ye, M. Cao, X. He, P. Li, K. Hu, L. Rao, S. M. Wu, H. B. Xiao and E. Gao: Detection of survivin-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. *Ann Surg Oncol*, 15(11), 3073-82 (2008)

65. Dalal K. M., Y. Woo, K. Kelly, C. Galanis, M. Gonen, Y. Fong and D. G. Coit: Detection of micrometastases in peritoneal washings of gastric cancer patients by the reverse transcriptase polymerase chain reaction. *Gastric Cancer*, 11(4), 206-13 (2008)

66. Maraculla T ., F.J. Ramos and I. Tabernero:. Aurora Kinase family: a new target for anticancro drug. *Recent Pat Anticancer Drug Discov;* 3: 114-22 (2008)

67. Ju H., H. Cho, Y. S. Kim, W. H. Kim, C. Ihm, S. M. Noh, J. B. Kim, D. S. Hahn, B. Y. Choi and C. Kang: Functional polymorphism 57Val>Ile of aurora kinase A associated with increased risk of gastric cancer progression. *Cancer Lett,* 242(2), 273-9 (2006)

68. Lazar D., S. Taban, C. Ardeleanu, C. Simionescu, I. Sporea, M. Cornianu and C. Vernic: Immunohistochemical expression of the cyclooxygenase-2 (COX-2) in gastric cancer. The correlations with the tumor angiogenesis and patients' survival. *Rom J Morphol Embryol,* 49(3), 371-9 (2008)

69. Walduck A. K., M. Weber, C. Wunder, S. Juettner, M. Stolte, M. Vieth, B. Wiedenmann, T. F. Meyer, M. Naumann and M. Hoecker: Identification of novel cyclooxygenase-2-dependent genes in Helicobacter pylori infection in vivo. *Mol Cancer*, 8, 22 (2009)

70. Song S. Y., H. J. Son, M. H. Kim, E. S. Nam, J. C. Rhee and C. Park: Prognostic significance of maspin expression in human gastric adenocarcinoma. *Hepatogastroenterology*, 54(75), 973-6 (2007)

71. Mori K., T. Suzuki, H. Uozaki, H. Nakanishi, T. Ueda, Y. Matsuno, Y. Kodera, H. Sakamoto, N. Yamamoto, M. Sasako, M. Kaminishi and H. Sasaki: Detection of minimal gastric cancer cells in peritoneal washings by focused microarray analysis with multiple markers: clinical implications. *Ann Surg Oncol*, 14(5), 1694-702 (2007)

Key Words: Gastric cancer, Metastasis, Markers, Screening, Follow Up, Review

Send correspondence to: Alessandro Cappellani, University of Catania, Policlinico , Via Santa Sofia 84, 95123 Catania (CT), Italy, Tel: 39-095-3782212; Fax: 39-095-335146, E-mail: alecap@unict.it

http://www.bioscience.org/current/volS2.htm