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REVIEW

Genomic and genetic alterations influence the progression of gastric cancer

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Abstract

Gastric cancer is one of the leading causes of cancer-related deaths worldwide, although the incidence has gradually decreased in many Western countries. Two main gastric cancer histotypes, intestinal and diffuse, are recognised. Although most of the described genetic alterations have been observed in both types, different genetic pathways have been hypothesized. Genetic and epigenetic events, including 1q loss of heterozygosity (LOH), microsatellite instability and hypermethylation, have mostly been reported in intestinal-type gastric carcinoma and its precursor lesions, whereas 17p LOH, mutation or loss of E-cadherin are more often implicated in the development of diffuse-type gastric cancer. In this review, we summarize the sometimes contradictory findings regarding those markers which influence the progression of gastric adenocarcinoma.

Key words: Gastric cancer; Gene alterations; Prognosis; Molecular pathology

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INTRODUCTION

Gastric cancer is one of the leading causes of cancer-related deaths worldwide, although the incidence has gradually decreased in many Western countries[1]. Several attempts to classify gastric cancer have been made over the past decades. Most successful, and widely used, is the classification by Lauren, which, by microscopic morphology alone, distinguishes two main cancer pathogeneses, diffuse and intestinal subtypes, which clearly appear as dissimilar clinical and epidemiological entities. Although most of the genetic alterations that have been reported are observed in both intestinal and diffuse gastric cancers, it has become apparent that these two tumor types result from different genetic pathways[2] (Table 1).

Microsatellite instability and *p53* mutation, reduced

p27 expression, cyclin E overexpression and 6.0-kb transcripts of the *c-met* gene are involved in malignant transformation from precancerous lesions to intestinal-type gastric cancer. In addition, *DCC* loss, *APC* mutations, 1q loss of heterozygosity (LOH), *p27* loss, reduced expression of tumor growth factor (TGF)-β type I receptor and *HER2* gene amplification are frequently associated with an advanced stage of intestinal-type gastric carcinoma. In contrast, LOH at chromosome 17p (*p53*) and mutation or loss of E-cadherin are more often implicated in the development of diffuse-type gastric cancer, while loss of *p27* and gene amplification of *K-sam* and *c-met* lead to disease progression and metastatic spread.

The two types of gastric carcinoma organize different patterns of interplay between neoplastic and stromal cells through the growth factor/cytokine receptor system, which has a critical role in cell growth, apoptosis, morphogenesis, angiogenesis and metastasis. Other genetic factors, such as DNA polymorphism and genetic instability, may also be implicated in the two distinct major genetic pathways of gastric carcinogenesis.

GENOMIC INSTABILITY

Two phenotypes of genomic instability are generally recognized in gastric cancer: the phenotype associated with microsatellite instability (MSI) and that which is associated with chromosomal instability (CIN). These phenotypes are not necessarily independent and may even overlap in some cases[3].

MSI

MSI is a common feature of gastric cancer due to a deficit in the DNA mismatch repair system and derives from the presence of spontaneous DNA replication errors in simple repetitive sequences[4]. A standard panel of microsatellite markers, including mononucleotide (*BAT26* and *BAT25*) and dinucleotide (*D2S123*, *D5S346* and *D17S250*) repeats, has been recommended and guidelines for MSI testing (Bethesda Guidelines) have been drawn up[5]. Using the reference panel, three levels of MSI can be identified: high-level MSI (MSI-H), low-level MSI (MSI-L) and microsatellite stable (MSS). Recently, it has been established that mononucleotide repeats are instrumental in detecting MSI-H tumors because of their high sensitivity and specificity, and MSI-L has been defined as instability limited to dinucleotide loci[6]. After the adoption of the Bethesda panel, MSI-H phenotype was reported in a range of 5%-50% of all gastric carcinomas with significant differences in various ethnic groups. MSI-H appears to be a phenotypical marker of an underlying cellular defect involving DNA mismatch repair (MMR). Functional inactivation by mutations or epigenetic mechanisms of MMR genes, including *hMLH1* and *hMSH2*, is responsible for the MSI-H phenotype in gastric cancer. Abnormal loss of protein expression of either hMLH1 or hMSH2 has been observed in MSI-H gastric carcinomas[7]. In particular, altered expression of hMLH1 has been associated with gene inactivation by promoter hypermethylation.

MSI-H gastric carcinomas follow a molecular pathway of tumor progression, characterized by the presence of multiple frameshift mutations affecting mononucleotide tracts within genes involved in cancer-related molecular networks which control cellular homeostasis at different levels. MSI-related mutations occur in many genes at variable frequencies[4]. Genes regulating cell-cycle and apoptotic signaling are frequently targeted in MSI-H gastric carcinomas and include *TGFβRII*, *IGFIIR*, *TCF4*, *RIZ*, *BAX*, *CASPASE5*, *FAS*, *BCL10* and *APAF1*[8]. Moreover, genes involved in genomic integrity maintenance, i.e. *hMSH6*, *hMSH3*, *MED1*, *RAD50*, *BLM*, *ATR* and *MRE11*, are also frequently altered in MSI-H tumors[9]. Several studies indicate that, in most MSI-H gastric cancers, multiple target genes are simultaneously mutated and multiple hits impact on different genes in the same pathway[10]. In contrast, gastric carcinomas with MSS and MSI-L exhibit predominant p53 mutations[7].

As compared with MSS or MSI-L, gastric carcinomas with MSI-H show a significantly higher frequency of antral location, intestinal subtype, a lower incidence of lymph node metastasis and improved survival[8,11-15].

CIN

CIN is a feature of various tumors, including gastric cancer, commonly associated with chromosomal aberrations responsible for major modifications of DNA content, i.e. changes in chromosome copy number, and also high-level LOH, gene deletions and/or amplifications[16,17]. All these alterations may lead to oncogene activation and/or tumor suppressor gene inactivation. As with other tumors, aneuploidy is generally considered an unfavorable prognostic factor[18-21], though contrasting results have been reported[22-25].

High CIN levels have also been associated with a shorter survival in gastric cancer patients[26] and high LOH frequencies have been identified at several chromosome arms, including 1p, 3p, 4p, 5q, 7p, 8p, 8q, 9p, 12p, 13q, 17p, 18q, 20q and 22q[27-29].

The allelotype of gastric carcinoma is similar to that of colorectal and esophageal cancers, suggesting the presence of a common genetic pathway for tumor development. Some of these chromosomal segments include genes which are strongly implicated in carcinogenesis, such as the *p53* gene on chromosome 17, *DCC*, *DPC4* and *SMAD2* genes on chromosome 18, and *APC* and *MCC* genes on chromosome 5. Several studies have found that tumors with LOH at chromosome 5q, 18q or 17p had a poorer prognosis than tumors that did not show LOH at these sites[30,31].

EPIGENETIC INSTABILITY

Epigenetic changes, such as aberrant methylation of CpG islands in promoter regions, are commonly detected in human cancers and can permanently inactivate tumor-suppressor genes and affect important pathways of cell cycle regulation and proliferation. The methylation of CpG islands may be considered a third molecular phenotype of

Table 1 Molecular genetic changes in gastric cancer

	Abnormalities	Intestinal phenotype (%)	Diffuse phenotype (%)	Local progression	Distant metastasis	Prognosis	Prolonged survival	Ref.
Microsatellite instability	Mutation, hypermethylation, reduced expression	20-30	0-10	No	NA	Good	Yes	[8,11]
Tyrosine kinases								
HER2/neu	Amplification/overexpression	10-15	0	Yes	Yes	Poor	No	[57-59]
RUNX3	Hemizygous deletion/ hypermethylation/loss of expression	15-45	40-80	Yes	Yes	Poor	No	[62-64]
FHIT	Loss of protein expression (LOH, MSI)	35-65	20-80	Yes	Yes	Poor	No	[65,66]
NM23	Downregulation	3-25	30-70	Yes	Yes	Poor	Discordant results	[73,75]
VEGF	Overexpression	65	35-45	Yes	Yes	Poor	No	[77-79]
HIF-1α	Overexpression	25-60	45-60	Discordant results	NA	NA	Discordant results	[80-82]
COX2	Overexpression	60-70	30-70	Yes	Yes	Discordant results	No	[77,83,84]
SPARC	Overexpression	70-80	25-55	Discordant results	Yes	Poor	No	[85-87]
p53	LOH/mutation/hypermethylation/ overexpression	20-40	20-40	Yes	Yes	No correlation	No	[88-91]
p21	Loss	60		Yes	Yes	Poor	No	[92-94]
p27	Reduced expression	50		Yes	Yes	Poor	No	[95-97]
bcl2	LOH/overexpression	40	0	No	No	Good	Yes	[98]
BAX	Reduced expression	10	5	NA	Yes	Poor	No	[99]
pRb	Reduced expression	60	50	NA	NA	Poor	No	[1,92]
c-myc	Overexpression	45	10	Possible	Possible	Poor	No	[101-104]
	Amplification	15	5	Possible	Possible	Poor	No	
Cyclin E	Amplification/overexpression	15-20		Yes	Yes	Poor	No	[92]
E-cadherin	LOH/mutation/hypermethylation/ reduced expression	0	50	Yes	Yes	Poor	No	[107,108]
MUC1	Overexpression	30-60	15-65	Yes	No	Poor	No	[65,106,110]
PRL-3	Overexpression	30-40	25-60	Yes	Discordant results	Poor	No	[112-114]
Tumor-associated proteases								[115-117]
PAI-1	Overexpression	45-75	35-50	Yes	Yes	Poor	No	
uPAR	Overexpression	40-75	30-50	Yes	NA	Poor	No	
uPA	Overexpression	65	30	Yes	NA	Poor	No	

FHIT: Fragile histidine triad; LOH: Loss of heterozygosity; MSI: Microsatellite instability; VEGF: Vascular endothelial growth factor; HIF-1α: Hypoxia inducible factor-1α; COX2: Cyclooxygenase-2; SPARC: Secreted protein acidic and rich in cysteine; PRL-3: Phosphatase regenerating liver 3; PAI-1: Plasminogen activator inhibitor type Ⅰ; uPA: Urokinase-type plasminogen activator; u-PAR: u-PA receptor; NA: Not available.

gastric cancer and the tumor-related genes more commonly methylated are *APC*, *CDH1*, *MHL1*, *CDKN2A*, *CDKN2B* and *RUNX3*. It has also been widely reported that *CDKN2A*, *CDH1* and *MLH1* are more frequently inactivated by promoter methylation rather than by mutations[32].

A series of individual methylated genes has been related to prognosis in gastric cancer. Methylation of tumor-suppressor genes, such as *CDH1*[33], *DKK3*[34], *PTEN*[35] and *MGMT*[36], of putative tumor-suppressor genes, such as *TFPI2*[37] and *CACNA2D3*[38], and of other tumor-related genes, such as *PCDH10*[39] and *SOX2*[40], has been associated with shorter disease-free and/or overall survival.

The combined use of *APC* and *CDH1* methylation markers has identified a subgroup of patients with worse prognosis[41]. Conversely, methylation of single genes has been associated with a better prognosis in some cases. Patients showing methylation of *APC*[42], the M1 region of *MAL* promoter[43] and cyclooxygenase-2 (*COX2*)[44] showed prolonged survival, compared to patients without methylation of these genes.

As with colorectal cancer, the CpG island methylator phenotype (CIMP), characterized by concurrent promoter hypermethylation of multiple genes, has also been described in gastric cancer[45,46] and it has been shown to correlate with hypermethylation of other known cancer-related genes, such as *p16*, *hMLH1* and *THBS-1*[45,47]. Furthermore, the CIMP status is associated with clinically useful information and patients with negative CIMP methylation have significantly shorter survival than those with high CIMP methylation[46,48].

ALTERATIONS OF GENES INVOLVED IN MOLECULAR PATHWAYS

Genetic and genomic variations occurring in genes and molecules that participate in proliferation, invasion and metastasis (e.g. growth factors and their receptors, signal transducers, cell-cycle and apoptosis regulators, cell adhesion molecules, DNA repair genes and matrix metallo-

proteinases) may influence the prognosis of patients with gastric cancer.

Tyrosine kinases

Amplification of some tyrosine kinases (c-met, K-sam and HER2/neu) is associated with human gastric cancer progression. Alternatively, spliced transcripts and enhanced protein expression levels for some of these tyrosine kinases are correlated with the clinical outcome of gastric cancer patients[49].

The oncogene *c-met*, encoding for the hepatocyte growth factor receptor, is preferentially amplified in diffuse-type tumors and has been described to be well correlated with stage and prognosis[50,51]. Overexpression of c-met has also been shown to be associated with lower survival probability[52,53].

K-sam oncogene, a member of the fibroblast growth factor receptor family, is more frequently activated in diffuse-type tumors[2]. Overexpression of K-sam occurs in approximately 32% of diffuse-type gastric cancers, and the prognosis of K-sam-positive patients is poorer than that of K-sam-negative patients[54].

The HER2 protein (HER2/neu or ErbB-2) is a glycoprotein with tyrosine kinase activity, homologous to the epidermal growth factor receptor. HER2 is codified by a gene located on chromosome 17q21 and does not bind to any known ligand. Some studies demonstrated that overexpression of c-erbB2 is selectively found in intestinal tumors and may serve as a prognostic marker for tumor invasion and lymph node metastasis. Overexpression of HER2 protein in gastric cancer has been reported to range from 7.4% to 38%[55-57]. The prognostic value of HER2 expression and/or amplification has been widely investigated with controversial findings. Although most available studies indicate that the overexpression of HER2 is an independent prognostic factor associated with a shorter disease-free[58] and overall survival[57-59], some studies failed to confirm its prognostic role on multivariate analysis[51] or to find a correlation between HER2 overexpression and survival parameters[56,60]. Also associated with poor survival is the presence of HER2 amplification[61].

RUNX3

RUNX3, a gene that codifies for a member of the runt domain-containing family of transcription factors, frequently shows loss of expression due to hemizygous deletion and hypermethylation in gastric cancer. This gene, generally expressed in only 45%-50% of gastric cancer patients[62,63], positively regulates the expression of BIM and p21, and negatively regulates vascular endothelial growth factor (VEGF), thus affecting apoptosis, cell growth arrest and angiogenesis. The loss or substantial decrease of RUNX3 protein expression in gastric cancer has been significantly associated with shorter survival[62,64].

FHIT

The fragile histidine triad gene (*FHIT*) encodes a diadenosine 5',5'''-P1,P3-triphosphate hydrolase and is generally inactivated by deletion or methylation in several tumors, including gastric cancer. The absence of FHIT protein has been shown to correlate with higher tumor stage and histological grade[64], as well as with poor overall survival[65,66].

NM23

The *NM23* gene maps to chromosome 17q21 and encodes the nucleoside diphosphate kinase A, a member of the NDP kinase family. NM23 expression is reduced in metastatic melanoma and breast cancer cell lines[67]. Transfection into cell lines affects invasion, motility, colonization, differentiation and liver metastasis[68]. Decreased expression of NM23-H1, the human homologue, is found in advanced stages of human cancer[69,70].

The expression of the putative metastasis-suppressor gene *NM23* in gastric carcinoma is controversial. In several studies, expression of NM23 has been shown to be inversely correlated with the metastatic potential of gastric cancer[71,72] and with prolonged overall survival[73]. The results of other studies, however, suggest that *NM23* is not a metastasis suppressor gene and does not show correlation with metastasis[74,75].

VEGF

VEGF is a pro-angiogenic factor, frequently overexpressed in tumors. Mutations of *p53*, which under physiological conditions downregulates VEGF, may be responsible for its overexpression[76].

A correlation of the expression of VEGF with lymph node and liver metastasis has been described[77] and patients with VEGF-positive tumors have a rather worse prognosis than those with VEGF-negative tumors[78,79].

HIF-1α

The hypoxia inducible factor, HIF-1α, is a transcription factor that plays an essential role in cellular and systemic homeostatic responses to hypoxia. The prognostic role of HIF-1α expression in gastric cancer patients is controversial: high levels have been associated with a shorter overall survival[80], but also with no difference in survival parameters[81]. However, its upregulation (high HIF-1α mRNA or protein levels) has been found to be positively correlated with VEGF[82] or p53[80] protein expression in gastric cancer patients, and overall survival of patients with high mRNA levels of HIF-1α and VEGF, as well as of HIF-1α and p53, was shorter compared to patients with different features.

COX2

COX2 is one of the key isoenzymes in the production of prostaglandins, and is thought to be involved in carcinogenesis. Some studies indicate that COX2 may play a role in the development of gastric cancer, and its overexpression is associated with nodal metastasis, tumor invasion and differentiation, implicating a poor prognosis[77,83,84].

SPARC

The secreted protein acidic and rich in cysteine (SPARC or osteonectin) is a member of a family of matricellular

proteins that modulates cell-matrix interactions and cell function without participating in the structural scaffolding of the extracellular matrix. Since SPARC alters membrane permeability, cell shape, proliferation, migration and attachment, it may play a role in angiogenesis. It has been reported that its overexpression correlates with distant metastasis and poor prognosis[85-87]. It is not clear whether SPARC overexpression is a useful marker in the prediction of lymph node metastasis development[85].

p53

The p53 protein plays a fundamental role in cell growth and division. The function of the *p53* gene is more frequently altered due to LOH and mutation than to DNA methylation. Mutations of *p53* are present in about 40% of early and advanced, well-differentiated gastric cancers[88]. A lower incidence of *p53* mutations has been shown in young patients compared to older patients[89].

p53 can be investigated by immunohistochemical techniques, bearing in mind that the half-life of the p53 mutant protein is prolonged. Cells carrying the p53 mutant protein can be stained with antibodies against p53, whereas cells carrying normal p53 are negative. Sequencing of the gene after screening can also be performed in order to determine the mutation location within the gene[90].

Overexpression of p53 often occurs in the early stages of intestinal-type tumors, and there is no significant difference between early and advanced cancers. In contrast, p53 abnormalities are not often seen in the early stages of diffuse-type tumors, but tend to occur as the disease progresses[91].

p21

p53 cell cycle regulatory function is mediated by different effectors. One of these is a cyclin-dependant kinase inhibitor (CDK I), the p21 protein. The cell cycle check points are controlled by a cascade of phosphorylation. Protein kinases such as cyclin-dependent kinases are activated by cyclins and inhibited by CDK I, although p21 is up-regulated not only through a p53 pathway, but also through a TGFβRII pathway.

Levels of p21 expression could indicate the absence of a functional p53 protein in neoplastic cells. It has been reported that the survival of gastric cancer patients with p21-positive tumors is significantly longer than that of patients with p21-negative tumors[92]. The expression of p21 is usually assessed in combination with p53 status and contributes to predicting the clinical outcome of gastric cancer patients[93,94].

p27

It has been suggested that the cyclin-dependent inhibitor p27, which controls the transition from G1 to S in the cell cycle, has prognostic relevance in gastric cancer. Reduced p27 expression is detected in approximately 40%-50% of gastric cancers[28]. Some studies have shown that tumors with a low expression of p27 protein are poorly differentiated and at an advanced stage[95,96]. However, some authors have found no difference in overall survival of gastric cancer patients whether with high or low p27 expression[97]. p53, p21 and p27 have also been analyzed in combination, confirming their role as prognostic markers[91].

BCL2

BCL2 and *p53* are closely linked in the regulation of apoptosis. LOH at the *BCL2* locus is frequently observed in gastric cancer. The overexpression of BCL2 may have a role in the development of gastric cancers. It has been shown that BCL2 overexpression reduces cellular proliferative activity and correlates with a less aggressive biological behavior of the tumor. The prognostic role of BCL2 on its own or in association with p53 has not yet been elucidated[98].

BAX

BAX gene encodes a protein belonging to the BCL family members. Negative BAX protein expression has been associated with de-differentiation, lymph node metastasis and shorter survival, suggesting that BAX status may play a role in the development and differentiation of gastric cancer and tumor progression[99].

pRb

pRb encodes a protein that is a negative regulator of the cell cycle. Poor prognosis of gastric cancer patients with low levels of pRb expression has been reported[92,100].

c-myc

c-myc gene encodes a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. The c-myc protein has been shown to be significantly enhanced in well-differentiated gastric cancer[101] and associated with a poor prognosis[102]. Although c-myc is a short-lived protein in normal cells, its stability is increased in transformed cells through several mechanisms. One of these has recently been identified in the overexpression of a human oncoprotein, the cancerous inhibitor of protein phosphate 2A (CIP2A) that stabilizes c-myc[103]. Interestingly, the expression of CIP2A has been associated with reduced overall survival in gastric cancer patients[104].

Cyclin E

Cyclin E overexpression correlates with invasiveness and proliferation and may be a marker of tumor aggressiveness. Although somatic mutations of the cell cycle inhibitor $p16^{MTSI}$ are rare, its reduced expression is associated with depth of invasion and metastatic potential in both diffuse- and intestinal-type gastric carcinomas. However, recent data show that the survival of gastric cancer patients with cyclin E-positive tumors is not significantly shorter than that of negative patients[92].

E-cadherin

Cell adhesion molecules are implicated in human carcinogenesis. Cadherin is a superfamily of calcium-mediated membrane glycoproteins, forming one of the four classes of adhesion molecules. E-cadherin, one of the members

of the trasmembrane glycoprotein family expressed by epithelial tissues, not only acts as a cell adhesion molecule, but also plays an important role in growth development and carcinogenesis. The intact function of E-cadherin is crucial for the establishment and maintenance of epithelial tissue polarity and structural integrity. Around 25%-40% of hereditary diffuse gastric cancers are caused by heterozygous E-cadherin. The inactivation of the second allele occurs by mutation and methylation events, and this results in the complete inactivation of the protein[105]. Reduced expression of E-cadherin correlates with infiltrative and metastatic ability in gastric cancer[33] and the gene encoding E-cadherin, *CDH1*, was among the first to be considered as an invasion suppressor gene. Patients with E-cadherin-positive gastric cancers showed statistically significant prolonged 3- and 5-year survival rates, compared to patients with E-cadherin-negative tumors[33,106].

It has been shown that serum soluble E-cadherin is increased in several non-neoplastic diseases and also in various cancers, including gastric tumors. E-cadherin may be a potentially useful prognostic marker and high levels of soluble E-cadherin correlate with the depth of tumor invasion, as well as inoperability[107]. In addition, levels higher than 10000 ng/mL predict a survival of less than 3 years in more than 90% of patients[108].

The Wnt-frizzled-β-catenin signaling pathway is frequently activated in gastric carcinoma (e.g. upregulation of *Wnt* gene expression or of genes for Wnt ligand receptors, upregulation of *RAC1* and inactivation of *APC*), leading to poor differentiation and increased tumor invasiveness[109].

MUC1

Mucins are high-molecular weight glycoproteins containing oligosaccharides. These glycoproteins constitute the major components of the mucus that protects the gastric epithelium. Overexpression of mucin 1 (MUC1) has been linked to poor prognosis in gastric cancer patients[65,110].

It has been reported that MUC1 may accelerate tumor invasion by the impairment of E-cadherin[111]. The combined expression of MUC1 and E-cadherin shows that survival for gastric cancer patients with abnormal E-cadherin/MUC-positive expression was shorter than for patients with other expression patterns[106].

PRL-3

The phosphatase regenerating liver 3 (*PRL-3*) gene encodes a protein belonging to a class of prenylated protein tyrosine phosphatases. These proteins are cell signaling molecules with a regulatory role in several cellular processes. The prognostic role of PRL-3 in solid tumors, including gastric cancer, has been recently reviewed by Bessette *et al*[112]. High expression of PRL-3 has been associated with several unfavorable clinical parameters, such as tumor size, depth of invasion, lymphatic invasion, advanced stage and shorter overall survival. Successive studies have confirmed these findings[113,114].

Tumor-associated proteases

Tumor-associated proteases and their inhibitors play a central role in tumor invasion and metastasis. The positive correlation of histological data with the urokinase-type plasminogen activator (uPA) and the plasminogen activator inhibitor type I (PAI-1) has been reported. Moreover, the independent prognostic impact of both uPA and PAI-1 on the survival of gastric cancer patients has been demonstrated. Elevated uPA and PAI-1 levels have been shown to be associated with shorter survival[115,116]. A trend towards poor prognosis has also been observed in patients with high expression of the u-PA receptor (u-PAR)[113] and the uPA system may therefore be a target for novel therapeutic agents.

The prognostic role of some uPA genotypes has recently been investigated and an association was demonstrated between the exon 6 C/T polymorphism with invasive phenotype, but not with susceptibility or survival[117].

CONCLUSION

Gastric carcinomas are histologically and genetically heterogeneous and are influenced by gene-environment interactions resulting in the activation of multiple molecular pathways. The molecular subtypes of gastric cancer include three main groups of tumors characterized by either the CIN, the MSI or the CIMP pathways. Currently, it is not clear whether or in what way knowledge of these subtypes of gastric carcinomas is of use in clinical practice, with regard to predicting specific pathways with mutational and regulatory alterations that may interfere with targeted therapies.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249
2 **Tahara E**. Genetic pathways of two types of gastric cancer. *IARC Sci Publ* 2004; 327-349
3 **Hiyama T**, Tanaka S, Yoshihara M, Sasao S, Kose K, Shima H, Tuncel H, Ueno Y, Ito M, Kitadai Y, Yasui W, Haruma K, Chayama K. Chromosomal and microsatellite instability in sporadic gastric cancer. *J Gastroenterol Hepatol* 2004; **19**: 756-760
4 **Imai K**, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis* 2008; **29**: 673-680
5 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257
6 **Gologan A**, Krasinskas A, Hunt J, Thull DL, Farkas L, Sepulveda AR. Performance of the revised Bethesda guidelines for identification of colorectal carcinomas with a high level of microsatellite instability. *Arch Pathol Lab Med* 2005; **129**: 1390-1397
7 **Simpson AJ**, Caballero OL, Pena SD. Microsatellite instability as a tool for the classification of gastric cancer. *Trends Mol Med* 2001; **7**: 76-80
8 **Iacopetta BJ**, Soong R, House AK, Hamelin R. Gastric carcinomas with microsatellite instability: clinical features and mutations to the TGF-beta type II receptor, IGFII receptor, and BAX genes. *J Pathol* 1999; **187**: 428-432
9 **Ottini L**, Falchetti M, Saieva C, De Marco M, Masala G,

Zanna I, Paglierani M, Giannini G, Gulino A, Nesi G, Mariani Costantini R, Palli D. MRE11 expression is impaired in gastric cancer with microsatellite instability. *Carcinogenesis* 2004; **25**: 2337-2343

10 **Kim MS**, Kim SS, Ahn CH, Yoo NJ, Lee SH. Frameshift mutations of Wnt pathway genes AXIN2 and TCF7L2 in gastric carcinomas with high microsatellite instability. *Hum Pathol* 2009; **40**: 58-64

11 **Falchetti M**, Saieva C, Lupi R, Masala G, Rizzolo P, Zanna I, Ceccarelli K, Sera F, Mariani-Costantini R, Nesi G, Palli D, Ottini L. Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival. *Hum Pathol* 2008; **39**: 925-932

12 **Hayden JD**, Cawkwell L, Quirke P, Dixon MF, Goldstone AR, Sue-Ling H, Johnston D, Martin IG. Prognostic significance of microsatellite instability in patients with gastric carcinoma. *Eur J Cancer* 1997; **33**: 2342-2346

13 **Yamamoto H**, Perez-Piteira J, Yoshida T, Terada M, Itoh F, Imai K, Perucho M. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. *Gastroenterology* 1999; **116**: 1348-1357

14 **Choi SW**, Choi JR, Chung YJ, Kim KM, Rhyu MG. Prognostic implications of microsatellite genotypes in gastric carcinoma. *Int J Cancer* 2000; **89**: 378-383

15 **Corso G**, Pedrazzani C, Marrelli D, Pascale V, Pinto E, Roviello F. Correlation of microsatellite instability at multiple loci with long-term survival in advanced gastric carcinoma. *Arch Surg* 2009; **144**: 722-727

16 **Sugai T**, Habano W, Jiao YF, Suzuki M, Takagane A, Nakamura S. Analysis of genetic alterations associated with DNA diploidy, aneuploidy and multiploidy in gastric cancers. *Oncology* 2005; **68**: 548-557

17 **Sánchez-Pérez I**, García Alonso P, Belda Iniesta C. Clinical impact of aneuploidy on gastric cancer patients. *Clin Transl Oncol* 2009; **11**: 493-498

18 **Yonemura Y**, Ooyama S, Sugiyama K, Kamata T, De Aretxabala X, Kimura H, Kosaka T, Yamaguchi A, Miwa K, Miyazaki I. Retrospective analysis of the prognostic significance of DNA ploidy patterns and S-phase fraction in gastric carcinoma. *Cancer Res* 1990; **50**: 509-514

19 **Rugge M**, Sonego F, Panozzo M, Baffa R, Rubio J Jr, Farinati F, Nitti D, Ninfo V, Ming SC. Pathology and ploidy in the prognosis of gastric cancer with no extranodal metastasis. *Cancer* 1994; **73**: 1127-1133

20 **Danesi DT**, Spanò M, Fabiano A, Altavista P, Pasqualetti P, Toscano MG, Antonini F, Catalano P, Mecozzi A, Picconi A, Daffinà A, Cucchiara G. Flow cytometric DNA ploidy, p53, PCNA, and c-erbB-2 protein expressions as predictors of survival in surgically resected gastric cancer patients. *Cytometry* 2000; **42**: 27-34

21 **Wiksten JP**, Lundin J, Nordling S, Kokkola A, Haglund C. Comparison of the prognostic value of a panel of tissue tumor markers and established clinicopathological factors in patients with gastric cancer. *Anticancer Res* 2008; **28**: 2279-2287

22 **Ohyama S**, Yonemura Y, Miyazaki I. Proliferative activity and malignancy in human gastric cancers. Significance of the proliferation rate and its clinical application. *Cancer* 1992; **69**: 314-321

23 **Lee KH**, Lee JS, Lee JH, Kim SW, Suh C, Kim WK, Kim SH, Min YI, Kim BS, Park KC, Lee MS, Sun HS. Prognostic value of DNA flow cytometry in stomach cancer: a 5-year prospective study. *Br J Cancer* 1999; **79**: 1727-1735

24 **Sendler A**, Gilbertz KP, Becker I, Mueller J, Berger U, Fink U, van Beuningen D, Siewert JR. Proliferation kinetics and prognosis in gastric cancer after resection. *Eur J Cancer* 2001; **37**: 1635-1641

25 **Nesi G**, Bruno L, Saieva C, Caldini A, Girardi LR, Zanna I, Rapi S, Bechi P, Cortesini C, Palli D. DNA ploidy and S-phase fraction as prognostic factors in surgically resected gastric carcinoma: a 7-year prospective study. *Anticancer Res* 2007; **27**: 4435-4441

26 **Suzuki K**, Ohnami S, Tanabe C, Sasaki H, Yasuda J, Katai H, Yoshimura K, Terada M, Perucho M, Yoshida T. The genomic damage estimated by arbitrarily primed PCR DNA fingerprinting is useful for the prognosis of gastric cancer. *Gastroenterology* 2003; **125**: 1330-1340

27 **Kimura Y**, Noguchi T, Kawahara K, Kashima K, Daa T, Yokoyama S. Genetic alterations in 102 primary gastric cancers by comparative genomic hybridization: gain of 20q and loss of 18q are associated with tumor progression. *Mod Pathol* 2004; **17**: 1328-1337

28 **Panani AD**. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett* 2008; **266**: 99-115

29 **Buffart TE**, van Grieken NC, Tijssen M, Coffa J, Ylstra B, Grabsch HI, van de Velde CJ, Carvalho B, Meijer GA. High resolution analysis of DNA copy-number aberrations of chromosomes 8, 13, and 20 in gastric cancers. *Virchows Arch* 2009; **455**: 213-223

30 **Sanz-Ortega J**, Sanz-Esponera J, Caldes T, Gomez de la Concha E, Sobel ME, Merino MJ. LOH at the APC/MCC gene (5Q21) in gastric cancer and preneoplastic lesions. Prognostic implications. *Pathol Res Pract* 1996; **192**: 1206-1210

31 **Bamias AT**, Bai MC, Agnantis NJ, Michael MC, Alamanos YP, Stefanaki SV, Razi ED, Skarlos DV, Kappas AM, Pavlidis NA. Prognostic significance of the deleted in colorectal cancer gene protein expression in high-risk resected gastric carcinoma. *Cancer Invest* 2003; **21**: 333-340

32 **Ushijima T**, Sasako M. Focus on gastric cancer. *Cancer Cell* 2004; **5**: 121-125

33 **Graziano F**, Arduini F, Ruzzo A, Bearzi I, Humar B, More H, Silva R, Muretto P, Guilford P, Testa E, Mari D, Magnani M, Cascinu S. Prognostic analysis of E-cadherin gene promoter hypermethylation in patients with surgically resected, node-positive, diffuse gastric cancer. *Clin Cancer Res* 2004; **10**: 2784-2789

34 **Yu J**, Tao Q, Cheng YY, Lee KY, Ng SS, Cheung KF, Tian L, Rha SY, Neumann U, Röcken C, Ebert MP, Chan FK, Sung JJ. Promoter methylation of the Wnt/beta-catenin signaling antagonist Dkk-3 is associated with poor survival in gastric cancer. *Cancer* 2009; **115**: 49-60

35 **Kang YH**, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. *Lab Invest* 2002; **82**: 285-291

36 **Park TJ**, Han SU, Cho YK, Paik WK, Kim YB, Lim IK. Methylation of O(6)-methylguanine-DNA methyltransferase gene is associated significantly with K-ras mutation, lymph node invasion, tumor staging, and disease free survival in patients with gastric carcinoma. *Cancer* 2001; **92**: 2760-2768

37 **Jee CD**, Kim MA, Jung EJ, Kim J, Kim WH. Identification of genes epigenetically silenced by CpG methylation in human gastric carcinoma. *Eur J Cancer* 2009; **45**: 1282-1293

38 **Wanajo A**, Sasaki A, Nagasaki H, Shimada S, Otsubo T, Owaki S, Shimizu Y, Eishi Y, Kojima K, Nakajima Y, Kawano T, Yuasa Y, Akiyama Y. Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer. *Gastroenterology* 2008; **135**: 580-590

39 **Yu J**, Cheng YY, Tao Q, Cheung KF, Lam CN, Geng H, Tian LW, Wong YP, Tong JH, Ying JM, Jin H, To KF, Chan FK, Sung JJ. Methylation of protocadherin 10, a novel tumor suppressor, is associated with poor prognosis in patients with gastric cancer. *Gastroenterology* 2009; **136**: 640-651.e1

40 **Otsubo T**, Akiyama Y, Yanagihara K, Yuasa Y. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. *Br J Cancer* 2008; **98**: 824-831

41 **Leung WK**, To KF, Chu ES, Chan MW, Bai AH, Ng EK,

Chan FK, Sung JJ. Potential diagnostic and prognostic values of detecting promoter hypermethylation in the serum of patients with gastric cancer. *Br J Cancer* 2005; **92**: 2190-2194
42 **Ksiaa F**, Ziadi S, Amara K, Korbi S, Trimeche M. Biological significance of promoter hypermethylation of tumor-related genes in patients with gastric carcinoma. *Clin Chim Acta* 2009; **404**: 128-133
43 **Buffart TE**, Overmeer RM, Steenbergen RD, Tijssen M, van Grieken NC, Snijders PJ, Grabsch HI, van de Velde CJ, Carvalho B, Meijer GA. MAL promoter hypermethylation as a novel prognostic marker in gastric cancer. *Br J Cancer* 2008; **99**: 1802-1807
44 **de Maat MF**, van de Velde CJ, Umetani N, de Heer P, Putter H, van Hoesel AQ, Meijer GA, van Grieken NC, Kuppen PJ, Bilchik AJ, Tollenaar RA, Hoon DS. Epigenetic silencing of cyclooxygenase-2 affects clinical outcome in gastric cancer. *J Clin Oncol* 2007; **25**: 4887-4894
45 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; **59**: 5438-5442
46 **An C**, Choi IS, Yao JC, Worah S, Xie K, Mansfield PF, Ajani JA, Rashid A, Hamilton SR, Wu TT. Prognostic significance of CpG island methylator phenotype and microsatellite instability in gastric carcinoma. *Clin Cancer Res* 2005; **11**: 656-663
47 **Etoh T**, Kanai Y, Ushijima S, Nakagawa T, Nakanishi Y, Sasako M, Kitano S, Hirohashi S. Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers. *Am J Pathol* 2004; **164**: 689-699
48 **Kusano M**, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, Hosokawa M, Shinomura Y, Imai K, Tokino T. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island methylator phenotype and an association with Epstein-Barr virus. *Cancer* 2006; **106**: 1467-1479
49 **Lin W**, Kao HW, Robinson D, Kung HJ, Wu CW, Chen HC. Tyrosine kinases and gastric cancer. *Oncogene* 2000; **19**: 5680-5689
50 **Tsugawa K**, Yonemura Y, Hirono Y, Fushida S, Kaji M, Miwa K, Miyazaki I, Yamamoto H. Amplification of the c-met, c-erbB-2 and epidermal growth factor receptor gene in human gastric cancers: correlation to clinical features. *Oncology* 1998; **55**: 475-481
51 **Nakajima M**, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, Matsuda M, Sakaguchi T, Hirao T, Nakano H. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer* 1999; **85**: 1894-1902
52 **Huang TJ**, Wang JY, Lin SR, Lian ST, Hsieh JS. Overexpression of the c-met protooncogene in human gastric carcinoma--correlation to clinical features. *Acta Oncol* 2001; **40**: 638-643
53 **Drebber U**, Baldus SE, Nolden B, Grass G, Bollschweiler E, Dienes HP, Hölscher AH, Mönig SP. The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. *Oncol Rep* 2008; **19**: 1477-1483
54 **Toyokawa T**, Yashiro M, Hirakawa K. Co-expression of keratinocyte growth factor and K-sam is an independent prognostic factor in gastric carcinoma. *Oncol Rep* 2009; **21**: 875-880
55 **Gravalos C**, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008; **19**: 1523-1529
56 **Matsubara J**, Yamada Y, Hirashima Y, Takahari D, Okita NT, Kato K, Hamaguchi T, Shirao K, Shimada Y, Shimoda T. 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* 2008; **14**: 3022-3029
57 **Zhang XL**, Yang YS, Xu DP, Qu JH, Guo MZ, Gong Y, Huang J. Comparative study on overexpression of HER2/neu and HER3 in gastric cancer. *World J Surg* 2009; **33**: 2112-2118
58 **Allgayer H**, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. *J Clin Oncol* 2000; **18**: 2201-2209
59 **Yonemura Y**, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S, Miyazaki I, Endou Y, Tanaka M, Sasaki T. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. *Cancer Res* 1991; **51**: 1034-1038
60 **Sasano H**, Date F, Imatani A, Asaki S, Nagura H. Double immunostaining for c-erbB-2 and p53 in human stomach cancer cells. *Hum Pathol* 1993; **24**: 584-589
61 **Tanner M**, Hollmén M, Junttila TT, Kapanen AI, Tommola S, Soini Y, Helin H, Salo J, Joensuu H, Sihvo E, Elenius K, Isola J. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. *Ann Oncol* 2005; **16**: 273-278
62 **Hsu PI**, Hsieh HL, Lee J, Lin LF, Chen HC, Lu PJ, Hsiao M. Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer. *Ann Surg Oncol* 2009; **16**: 1686-1694
63 **Ogasawara N**, Tsukamoto T, Mizoshita T, Inada KI, Ban H, Kondo S, Takasu S, Ushijima T, Ito K, Ito Y, Ichinose M, Ogawa T, Joh T, Tatematsu M. RUNX3 expression correlates with chief cell differentiation in human gastric cancers. *Histol Histopathol* 2009; **24**: 31-40
64 **Wei D**, Gong W, Oh SC, Li Q, Kim WD, Wang L, Le X, Yao J, Wu TT, Huang S, Xie K. Loss of RUNX3 expression significantly affects the clinical outcome of gastric cancer patients and its restoration causes drastic suppression of tumor growth and metastasis. *Cancer Res* 2005; **65**: 4809-4816
65 **Lee HS**, Lee HK, Kim HS, Yang HK, Kim WH. Tumour suppressor gene expression correlates with gastric cancer prognosis. *J Pathol* 2003; **200**: 39-46
66 **Zhao P**, Liu W, Lu YL. Clinicopathological significance of FHIT protein expression in gastric adenocarcinoma patients. *World J Gastroenterol* 2005; **11**: 5735-5738
67 **Steeg PS**, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, Sobel ME. Evidence for a novel gene associated with low tumor metastatic potential. *J Natl Cancer Inst* 1988; **80**: 200-204
68 **Leone A**, Flatow U, King CR, Sandeen MA, Margulies IM, Liotta LA, Steeg PS. Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23-transfected melanoma cells. *Cell* 1991; **65**: 25-35
69 **Tokunaga Y**, Urano T, Furukawa K, Kondo H, Kanematsu T, Shiku H. Reduced expression of nm23-H1, but not of nm23-H2, is concordant with the frequency of lymph-node metastasis of human breast cancer. *Int J Cancer* 1993; **55**: 66-71
70 **Mandai M**, Konishi I, Koshiyama M, Mori T, Arao S, Tashiro H, Okamura H, Nomura H, Hiai H, Fukumoto M. Expression of metastasis-related nm23-H1 and nm23-H2 genes in ovarian carcinomas: correlation with clinicopathology, EGFR, c-erbB-2, and c-erbB-3 genes, and sex steroid receptor expression. *Cancer Res* 1994; **54**: 1825-1830
71 **Kodera Y**, Isobe K, Yamauchi M, Kondoh K, Kimura N, Akiyama S, Itoh K, Nakashima I, Takagi H. Expression of nm23 H-1 RNA levels in human gastric cancer tissues. A negative correlation with nodal metastasis. *Cancer* 1994; **73**: 259-265
72 **Nakayama H**, Yasui W, Yokozaki H, Tahara E. Reduced expression of nm23 is associated with metastasis of human

gastric carcinomas. *Jpn J Cancer Res* 1993; **84**: 184-190
73 **Lee KE**, Lee HJ, Kim YH, Yu HJ, Yang HK, Kim WH, Lee KU, Choe KJ, Kim JP. Prognostic significance of p53, nm23, PCNA and c-erbB-2 in gastric cancer. *Jpn J Clin Oncol* 2003; **33**: 173-179
74 **Müller W**, Schneiders A, Hommel G, Gabbert HE. Expression of nm23 in gastric carcinoma: association with tumor progression and poor prognosis. *Cancer* 1998; **83**: 2481-2487
75 **Nesi G**, Palli D, Pernice LM, Saieva C, Paglierani M, Kroning KC, Catarzi S, Rubio CA, Amorosi A. Expression of nm23 gene in gastric cancer is associated with a poor 5-year survival. *Anticancer Res* 2001; **21**: 3643-3649
76 **Scartozzi M**, Galizia E, Freddari F, Berardi R, Cellerino R, Cascinu S. Molecular biology of sporadic gastric cancer: prognostic indicators and novel therapeutic approaches. *Cancer Treat Rev* 2004; **30**: 451-459
77 **Tang H**, Wang J, Bai F, Zhai H, Gao J, Hong L, Xie H, Zhang F, Lan M, Yao W, Liu J, Wu K, Fan D. Positive correlation of osteopontin, cyclooxygenase-2 and vascular endothelial growth factor in gastric cancer. *Cancer Invest* 2008; **26**: 60-67
78 **Duff SE**, Li C, Jeziorska M, Kumar S, Saunders MP, Sherlock D, O'Dwyer ST, Jayson GC. Vascular endothelial growth factors C and D and lymphangiogenesis in gastrointestinal tract malignancy. *Br J Cancer* 2003; **89**: 426-430
79 **Vidal O**, Metges JP, Elizalde I, Valentíni M, Volant A, Molina R, Castells A, Pera M. High preoperative serum vascular endothelial growth factor levels predict poor clinical outcome after curative resection of gastric cancer. *Br J Surg* 2009; **96**: 1443-1451
80 **Sumiyoshi Y**, Kakeji Y, Egashira A, Mizokami K, Orita H, Maehara Y. Overexpression of hypoxia-inducible factor 1alpha and p53 is a marker for an unfavorable prognosis in gastric cancer. *Clin Cancer Res* 2006; **12**: 5112-5117
81 **Griffiths EA**, Pritchard SA, Valentine HR, Whitchelo N, Bishop PW, Ebert MP, Price PM, Welch IM, West CM. Hypoxia-inducible factor-1alpha expression in the gastric carcinogenesis sequence and its prognostic role in gastric and gastro-oesophageal adenocarcinomas. *Br J Cancer* 2007; **96**: 95-103
82 **Ma J**, Zhang L, Ru GQ, Zhao ZS, Xu WJ. Upregulation of hypoxia inducible factor 1alpha mRNA is associated with elevated vascular endothelial growth factor expression and excessive angiogenesis and predicts a poor prognosis in gastric carcinoma. *World J Gastroenterol* 2007; **13**: 1680-1686
83 **Shi H**, Xu JM, Hu NZ, Xie HJ. Prognostic significance of expression of cyclooxygenase-2 and vascular endothelial growth factor in human gastric carcinoma. *World J Gastroenterol* 2003; **9**: 1421-1426
84 **Han SL**, Tang HJ, Hua YW, Ji SQ, Lin DX. Expression of COX-2 in stomach cancers and its relation to their biological features. *Dig Surg* 2003; **20**: 107-114
85 **Zhao ZS**, Wang YY, Chu YQ, Ye ZY, Tao HQ. SPARC is associated with gastric cancer progression and poor survival of patients. *Clin Cancer Res* 2010; **16**: 260-268
86 **Wang CS**, Lin KH, Chen SL, Chan YF, Hsueh S. Overexpression of SPARC gene in human gastric carcinoma and its clinic-pathologic significance. *Br J Cancer* 2004; **91**: 1924-1930
87 **Junnila S**, Kokkola A, Mizuguchi T, Hirata K, Karjalainen-Lindsberg ML, Puolakkainen P, Monni O. Gene expression analysis identifies over-expression of CXCL1, SPARC, SPP1, and SULF1 in gastric cancer. *Genes Chromosomes Cancer* 2010; **49**: 28-39
88 **Uchino S**, Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S. p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int J Cancer* 1993; **54**: 759-764
89 **Rugge M**, Shiao YH, Busatto G, Cassaro M, Strobbe C, Russo VM, Leo G, Parenti AR, Scapinello A, Arslan P, Egarter-Vigl E. The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low but are associated with a cardiac location. *Mol Pathol* 2000; **53**: 207-210
90 **Shiao YH**, Palli D, Caporaso NE, Alvord WG, Amorosi A, Nesi G, Saieva C, Masala G, Fraumeni JF Jr, Rice JM. Genetic and immunohistochemical analyses of p53 independently predict regional metastasis of gastric cancers. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 631-633
91 **Liu XP**, Tsushimi K, Tsushimi M, Oga A, Kawauchi S, Furuya T, Sasaki K. Expression of p53 protein as a prognostic indicator of reduced survival time in diffuse-type gastric carcinoma. *Pathol Int* 2001; **51**: 440-444
92 **Kouraklis G**, Katsoulis IE, Theocharis S, Tsourouflis G, Xipolitas N, Glinavou A, Sioka C, Kostakis A. Does the expression of cyclin E, pRb, and p21 correlate with prognosis in gastric adenocarcinoma? *Dig Dis Sci* 2009; **54**: 1015-1020
93 **Seo YH**, Joo YE, Choi SK, Rew JS, Park CS, Kim SJ. Prognostic significance of p21 and p53 expression in gastric cancer. *Korean J Intern Med* 2003; **18**: 98-103
94 **Okuyama T**, Maehara Y, Kabashima A, Takahashi I, Kakeji Y, Sugimachi K. Combined evaluation of expressions of p53 and p21 proteins as prognostic factors for patients with gastric carcinoma. *Oncology* 2002; **63**: 353-361
95 **Sgambato A**, Migaldi M, Leocata P, Ventura L, Criscuolo M, Di Giacomo C, Capelli G, Cittadini A, De Gaetani C. Loss of p27Kip1 expression is a strong independent prognostic factor of reduced survival in N0 gastric carcinomas. *Cancer* 2000; **89**: 2247-2257
96 **Nitti D**, Belluco C, Mammano E, Marchet A, Ambrosi A, Mencarelli R, Segato P, Lise M. Low level of p27(Kip1) protein expression in gastric adenocarcinoma is associated with disease progression and poor outcome. *J Surg Oncol* 2002; **81**: 167-175; discussion 175-176
97 **Wiksten JP**, Lundin J, Nordling S, Kokkola A, von Boguslawski K, Haglund C. The prognostic value of p27 in gastric cancer. *Oncology* 2002; **63**: 180-184
98 **Lee HK**, Lee HS, Yang HK, Kim WH, Lee KU, Choe KJ, Kim JP. Prognostic significance of Bcl-2 and p53 expression in gastric cancer. *Int J Colorectal Dis* 2003; **18**: 518-525
99 **Anagnostopoulos GK**, Stefanou D, Arkoumani E, Chalkley L, Karagiannis J, Paraskeva K, Mathou N, Dellaporta E, Tsianos E, Agnantis NJ. Expression of Bax protein in gastric carcinomas. A clinicopathological and immunohistochemical study. *Acta Gastroenterol Belg* 2007; **70**: 285-289
100 **Feakins RM**, Nickols CD, Bidd H, Walton SJ. Abnormal expression of pRb, p16, and cyclin D1 in gastric adenocarcinoma and its lymph node metastases: relationship with pathological features and survival. *Hum Pathol* 2003; **34**: 1276-1282
101 **Sanz-Ortega J**, Steinberg SM, Moro E, Saez M, Lopez JA, Sierra E, Sanz-Esponera J, Merino MJ. Comparative study of tumor angiogenesis and immunohistochemistry for p53, c-ErbB2, c-myc and EGFr as prognostic factors in gastric cancer. *Histol Histopathol* 2000; **15**: 455-462
102 **Han S**, Kim HY, Park K, Cho HJ, Lee MS, Kim HJ, Kim YD. c-Myc expression is related with cell proliferation and associated with poor clinical outcome in human gastric cancer. *J Korean Med Sci* 1999; **14**: 526-530
103 **Junttila MR**, Westermarck J. Mechanisms of MYC stabilization in human malignancies. *Cell Cycle* 2008; **7**: 592-596
104 **Khanna A**, Böckelman C, Hemmes A, Junttila MR, Wiksten JP, Lundin M, Junnila S, Murphy DJ, Evan GI, Haglund C, Westermarck J, Ristimäki A. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. *J Natl Cancer Inst* 2009; **101**: 793-805
105 **Becker KF**, Keller G, Hoefler H. The use of molecular biology in diagnosis and prognosis of gastric cancer. *Surg Oncol* 2000; **9**: 5-11
106 **Ohno T**, Aihara R, Kamiyama Y, Mochiki E, Asao T, Kuwano H. Prognostic significance of combined expression of

MUC1 and adhesion molecules in advanced gastric cancer. *Eur J Cancer* 2006; **42**: 256-263

107 **Chan AO**, Lam SK, Chu KM, Lam CM, Kwok E, Leung SY, Yuen ST, Law SY, Hui WM, Lai KC, Wong CY, Hu HC, Lai CL, Wong J. Soluble E-cadherin is a valid prognostic marker in gastric carcinoma. *Gut* 2001; **48**: 808-811

108 **Chan AO**, Chu KM, Lam SK, Wong BC, Kwok KF, Law S, Ko S, Hui WM, Yueng YH, Wong J. Soluble E-cadherin is an independent pretherapeutic factor for long-term survival in gastric cancer. *J Clin Oncol* 2003; **21**: 2288-2293

109 **Stock M**, Otto F. Gene deregulation in gastric cancer. *Gene* 2005; **360**: 1-19

110 **Zhang HK**, Zhang QM, Zhao TH, Li YY, Yi YF. Expression of mucins and E-cadherin in gastric carcinoma and their clinical significance. *World J Gastroenterol* 2004; **10**: 3044-3047

111 **Kondo K**, Kohno N, Yokoyama A, Hiwada K. Decreased MUC1 expression induces E-cadherin-mediated cell adhesion of breast cancer cell lines. *Cancer Res* 1998; **58**: 2014-2019

112 **Bessette DC**, Qiu D, Pallen CJ. PRL PTPs: mediators and markers of cancer progression. *Cancer Metastasis Rev* 2008; **27**: 231-252

113 **Dai N**, Lu AP, Shou CC, Li JY. Expression of phosphatase regenerating liver 3 is an independent prognostic indicator for gastric cancer. *World J Gastroenterol* 2009; **15**: 1499-1505

114 **Wang Z**, Cai SR, He YL, Zhan WH, Chen CQ, Cui J, Wu WH, Wu H, Song W, Zhang CH, Peng JJ, Huang XH. High expression of PRL-3 can promote growth of gastric cancer and exhibits a poor prognostic impact on patients. *Ann Surg Oncol* 2009; **16**: 208-219

115 **Nekarda H**, Schmitt M, Ulm K, Wenninger A, Vogelsang H, Becker K, Roder JD, Fink U, Siewert JR. Prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in completely resected gastric cancer. *Cancer Res* 1994; **54**: 2900-2907

116 **Beyer BC**, Heiss MM, Simon EH, Gruetzner KU, Babic R, Jauch KW, Schildberg FW, Allgayer H. Urokinase system expression in gastric carcinoma: prognostic impact in an independent patient series and first evidence of predictive value in preoperative biopsy and intestinal metaplasia specimens. *Cancer* 2006; **106**: 1026-1035

117 **Wu CY**, Wu MS, Chen YJ, Chen HP, Chen CJ, Chang CS, Lin JT. Clinicopathological significance of urokinase-type plasminogen activator genotypes in gastric cancer. *Hepatogastroenterology* 2008; **55**: 1890-1894

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