REVIEW

Molecular Genetics of Gastric Adenocarcinoma

Alvin Kim Hock Eng[1], *MMed (Surgery), FRCSEd*, Oi Lian Kon[2], *FRCPC, MD*

[1]Department of General Surgery, Singapore General Hospital, Singapore
[2]Division of Medical Sciences, National Cancer Centre, Singapore

ABSTRACT

Gastric carcinoma is the second leading cause of cancer deaths in the world. Its aetiology is closely linked to the bacterial pathogen *Helicobacter pylori* which is believed to induce a state of chronic inflammation that predisposes to a cascade of molecular and cellular alterations leading to carcinogenesis. Although the exact process of gastric carcinogenesis has yet to be elucidated fully, the interaction of the genetic factors with environmental factors is likely to be a significant consideration. Numerous genes and molecular pathways have been discovered to be associated with gastric adenocarcinoma and more importantly, it is now becoming possible to use some of these as means of prognostication and targeted therapy. This review will outline our current understanding of the aetiology and molecular genetics of gastric adenocarcinoma and its current clinical applications.

Keywords: aetiology, cancer, carcinogenesis, Helicobacter, stomach

INTRODUCTION

Despite a major decline in incidence and mortality rates over the last 50 years, gastric cancer remains the fourth most common cancer and the second leading cause of cancer death in the world[1]. Gastric cancer in Singapore is the fourth most common malignancy and the third highest cause of cancer-related mortality in both males and females[2]. As gastric carcinoma is often diagnosed at a late stage, conventional treatment in the form of surgery, radiation and chemotherapy results in overall 5-year survival rates of less than 30%[3].

The rapid advances in genomic and molecular techniques in the last 2 decades have yielded new insights into the processes of gastric carcinogenesis. It is now known that different pathways lead to intestinal- and diffuse-type gastric cancer. The molecular heterogeneity of gastric cancer is also largely due to a constant and complex interplay of genetic and environmental factors. This review will attempt to summarise the major mechanisms and risk factors that contribute to the development of gastric adenocarcinoma.

PATHOLOGY

More than 90% of gastric cancers (GC) are adenocarcinomas with lymphomas and stromal tumours accounting for the rest. The majority of adenocarcinomas are sporadic although clustered cases can occur whether due to an inherited genetic mutation and/or shared environmental factors.

It is generally recognised that there are 2 main histological types of gastric carcinoma as first described in 1965[4]. The Lauren classification defines these as the intestinal type which is characterised by the metaplastic transformation of gastric-type mucosa into an intestinal type with abundant goblet cells; and, the diffuse type which comprises cells that have defective adhesion properties and typically appear as poorly differentiated signet ring cells. Both types may also co-exist thereby giving rise to a third entity of "mixed" pathology.

The intestinal type is the more common variant and it is usually associated with chronic atrophic gastritis and multifocal gastric atrophy which then

progresses to metaplasia followed by dysplasia before manifesting overt carcinoma[5]. In contrast, the diffuse type cancers do not show this histological and temporal patten of development. Intestinal type cancer is more frequent in older patients in high-risk endemic regions whereas the diffuse type has a higher incidence in younger patients in low-risk areas and indeed may sometimes be hereditary. This observation supports the notion that the "nurture" component predominates in intestinal type GC whereas the "nature" component is the stronger factor in diffuse-type GC[6].

AETIOLOGY

It is now generally accepted that gastric cancer arises from gene-environment interactions[7,8]. The degree of influence of genetic factors versus environmental factors in gastric carcinogenesis appears to depend on the pathological type of cancer. The multi-step progression of intestinal type cancer suggests that environmental factors have a greater role; and this is supported by its close association with risk factors such as high salt intake, smoked or nitrated foods, low intake of fruits and vegetables and *H. pylori* infection[9]. The pathogenesis of diffuse-type cancer is less well understood although it is also associated with *H. pylori* infection[10]. The existence of lineages of hereditary diffuse gastric cancer (HDGC) such as the family of Napoleon Bonaparte[11] indicates the likelihood that genetic factors are the major determinant in diffuse-type cancer. Several other inherited conditions such as familial adenomatous polyposis (FAP), hereditary non-polyposis colon cancer (HNPCC), Peutz-Jeghers syndrome, and Li-Fraumeni syndrome also have strong associations with gastric adenocarcinoma[12].

With the exception of genetic syndromes, by far the strongest established risk factor for gastric cancer is *H. pylori* infection. Approximately 70–80% of gastric cancers are associated with *H. pylori* infection[13]. Although *H. pylori* has been classified as a WHO Class I carcinogen since 1994, the exact mechanism underlying malignant transformation remains to be clarified. The complexity of the underlying processes is demonstrated in the divergent responses of individuals to *H. pylori* infection. The majority of patients exhibit a mild pangastritis; a second group develops antral gastritis and duodenitis with hyperchlorydia; and, a third group experience gastritis mainly in the body of the stomach which eventually leads to hypochlorydia and atrophic gastritis which are precursors of intestinal type gastric cancer[14]. The markedly differing responses to *H. pylori* are attributed to a combination of bacterial virulence factors, host genetic susceptibility and environmental conditions[15].

H. pylori strains that possess the *cag*A, *vac*A, and *bab*A virulence-associated genes carry the highest risk for gastric cancer[16]. The *cag*A gene induces activation of the ERK/MAP (extracellular signal-regulated/mitogen-activated protein) kinase cascade that leads to expression of the *c-fos* and *c-jun* proto-oncogenes[17]. CagA protein is phosphorylated by cellular tyrosine phosphatase. Phosphorylated *cag*A protein binds to SHP2 which eventually leads to IL-8 secretion thus activating neutrophils. CagA protein has also been demonstrated to be the first bacterial oncoprotein that acts in human cells. Phosphorylated *cag*A protein binds to and induces deregulation of SHP-tyrosine phosphatase, which itself is another oncoprotein involved in human malignancies[18,19]. *H. pylori* strains containing *vac*A induce vacuole formation and apoptosis in gastric epithelial cells leading to ulcer formation[20]. The BabA protein induces tighter adhesion to gastric epithelial cells leading to more severe disease[16].

The host inflammatory response to chronic *H. pylori* infection is a major component of intestinal type gastric carcinogenesis. Cytokines such as IL-1β and TNF-α are potent inhibitors of acid secretion and also affect gastrin secretion[18]. Allelic polymorphisms are known to affect the degree of cytokine response to infections and chronic inflammation. IL-1β gene polymorphisms have been associated with increased incidence of hypochlorydia and atrophic gastritis in first-degree relatives of British gastric cancer patients[19]. IL-10 is an anti-inflammatory cytokine that down-regulates IL-1β, TNF-α and other pro-inflammatory cytokines. A relative deficiency of IL-10 may result in a pronounced inflammatory response with a higher risk of gastric cancer[20]. Allelic polymorphisms of IL-8, a potent chemokine for neutrophils with effects on cell proliferation, have also been shown to be associated with gastric cancer[21].

The model for intestinal-type gastric carcinogenesis in a patient with *H. pylori* infection would therefore involve a genotype comprising pro-inflammatory cytokine alleles that eventually lead to a constantly inflamed and hypochlorydic gastric mucosa. The

bacterial effect on mucosal inflammation would be exacerbated by the presence of *cag*A, *vac*A, and *bab*A gene products in the resident *H. pylori* . Once gastric acidity has been reduced, other bacteria may proliferate in the stomach leading to increased oxidative activity and the production of nitrosamines which can inflict further DNA damage.

GENOMIC MECHANISMS OF GASTRIC CARCINOGENESIS

The genomic mechanisms that cause genetic alterations leading to gastric cancer are still incompletely understood. The genetic and epigenetic changes in gastric cancer appear to be largely due to 3 general mechanisms of genomic instability[9]. The chromosomal instability mechanism has been associated with mutations in genes such as *BRCA1* and *BRCA2* (double-strand break repair genes) that control the structural integrity of chromosomes[22,23]. Chromosomal rearrangements may be accompanied by deletions or gains of specific oncogenes or tumour suppressor genes leading to changes in their activity thereby promoting carcinogenesis[12,24]. Genes which are affected by chromosomal instability include *p53*, *VEGF*, and *CDH1*. Microsatellites are short DNA sequence repeats that occur throughout the genome. The microsatellite instability (MSI) mechanism in gastric cancer is characterised by hypermethylation (rather than mutation as in HNPCC) of one or more of the mismatch repair genes, such as *MLH1* or *MSH2*, leading to different number of DNA sequence repeats in tumour cells. This could result in frameshift mutations leading to errors in translation of DNA into proteins products. An example of a gene affected by MSI frameshift mutation is TGFBR2[27,28]. MSI-positive gastric cancers are more likely to be antral tumours, less prone to *p53* mutations and have a generally better prognosis[25]. A third mechanism involves epigenetic changes through methylation of cytosine-phosphate-guanine (CpG) islands (regions where cytosine and guanine bases are in sequence on the same strand of DNA). Abnormal methylation of CpG islands causes transcriptional silencing of genes such as *CDH1* or *MLH1* that could eventually lead to malignancy[26,27]. The prevalence and co-existence of these 3 types of genomic instability is variable between intestinal and diffuse gastric cancers and within each individual subtype.

GENETIC ALTERATIONS OF GASTRIC CARCINOGENESIS

The development of gastric cancer is attributed to the accumulation of multiple gene abnormalities. These genetic aberrations include oncogene activation, tumour suppressor gene inactivation and telomerase reactivation[28]. Although each gastric cancer may have multiple genetic defects, no specific sequence of changes has yet been identified as pathognomonic for gastric cancer; therefore most of these genetic alterations may in fact only be associated with, rather than actually be causal of, the pathogenesis of gastric cancer.

A variety of proto-oncogenes are activated in gastric cancer and there is considerable variation between diffuse and intestinal type tumours. The membrane receptor *c-erb*B-2 (*HER2/neu*) is over-expressed in approximately 20% of intestinal-type gastric cancers but not in diffuse type tumours[29]. It has been associated with a shorter disease-specific and overall survival as well as with liver metastases[30,31].The K-*sam* oncogene which encodes for a keratinocyte growth factor has also been shown to be involved in gastric carcinogenesis[32,33]. It is highly expressed in 53% of diffuse-type cancers but is notably normal in intestinal type tumours[34]. The marked difference in expression of *c-erb*B-2 and K-*sam* has raised the possibility that they may be useful as prognostic biomarkers of intestinal and diffuse type carcinomas respectively[35]. Another notable proto-oncogene that is frequently over-expressed in gastric cancer is *c-met* which encodes a tyrosine kinase receptor for hepatocyte growth factor. It is amplified in 19.2% of intestinal-type cancers and in 38.5% of diffuse-type cancers[36] and is generally associated with a poorer prognosis.

Many tumour suppressor genes have been shown to be inactivated in gastric cancers, of which the most notable are *p53*, *APC*, *CDH1*, *p16*, *FHIT*, *DCC* and *RUNX3*. The *p53* gene is frequently inactivated in gastric carcinoma by mutations, loss of heterozygosity (LOH) or deletions[14]. It is altered in intestinal metaplasia (37.5%), dysplasia (58.3%) and overt carcinoma (66.7%), of both the diffuse- and intestinal-type cancers[37,38]. The *p53* protein is essential in regulating the cell cycle by its ability to induce growth arrest, activate DNA repair proteins and initiate apoptosis. One defective allele of *p53* is inherited in the Li-Fraumeni syndrome in which several independent tumours typically

develop in early adulthood. Mutations of the *APC* gene on chromosome 5q are the hallmark of familial adenomatous polyposis coli and these patients have a higher lifetime risk of developing gastric cancer. Although *APC* gene mutation and LOH are seen in approximately 60% of sporadic intestinal-type cancers, they are rare in diffuse-type cancers[39]. Wild-type *APC* protein binds cytoplasmic β-catenin, preventing it from entering the nucleus where it acts as a transcription factor for genes that drive cell proliferation. The accumulation of cellular β-catenin from an *APC* defect or β-catenin gene (*CTNNB1*) mutation eventually leads to abnormal cellular growth and proliferation. The RUNX3 protein is part of a heterodimeric transcription factor complex that either activates or suppresses gene transcription. It operates downstream of the TGF-β signalling pathway and modulates apoptosis and cellular proliferation. Approximately 45–60% of gastric cancers exhibit loss of *RUNX3* expression due to gene deletion or promoter methylation[40,41].

The *CDH1* tumour suppressor gene codes for the protein E-cadherin which is a homophilic cell-cell adhesion glycoprotein. Loss of E-cadherin function results in increased cellular proliferation, tissue invasion and cellular migration to distant metastasis. *CDH1* mutations are not characteristic of intestinal-type gastric cancers but occur in 50% of sporadic diffuse-type carcinomas[42]. Germline mututions of *CDH1* account for up to one-third of families with hereditary diffuse gastric cancer (HDGC)[43]. Mutations in other genes (as yet unknown) are likely to be responsible for the other two-thirds. HDGC is inherited in an autosomal dominant manner with penetrance of approximately 80% and an average age of onset in the fourth decade of life[44]. Patients with *CDH1*-HDGC remain normal until the second allele is inactivated, which usually occurs via promoter methylation[45].

Telomeres are repetitive sequences of DNA (TTAGGG) at the ends of chromosomes that gradually shorten with each successive mitotic division in normal cells, thus limiting the number of times each cell may divide before it senesces[46]. Reactivation of telomerase in stem cells and malignant cells prevents telomere shortening by adding new copies of the TTAGGG tandem repeat to the ends of chromosomes[47,48]. Human telomerase reverse transcriptase (hTERT) is one of the components present in the telomerase complex. Elevated expression of hTERT has been detected in the nuclei of gastric cancer cells, suggesting that it has an important role in gastric carcinogenesis[49].

MOLECULAR FACTORS OF GASTRIC CANCER INVASION AND METASTASIS

Gastric cancer is a highly aggressive disease with rapid patterns of spread resulting in many patients being diagnosed at a late stage. The mechanisms for this aggressive phenotype remain poorly understood although it is probably attributable to genetic and molecular factors that control cell growth, differentiation and adhesion[28]. Although the early stages of carcinogenesis are likely initiated by the activation of oncogenes and the inactivation of tumour suppressor genes, it is thought that these dysfunctional activities are then aggravated by the development of a "stress" microenvironment such as hypoxia, acidosis and free radical production, thus advancing the carcinogenic process into uncontrolled tumour growth[50]. Some over-expressed transcription factors and growth factors include NF-κB, CDX1, CDX2, EGF, VEGF, EGFR and TGF-α[28]. The action of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) generates signalling molecules such as nitric oxide and prostaglandins that promote tumour angiogenesis, tumour progression and result in poorer patient survival[51,52].

GASTRIC CANCER STEM CELLS

Cancer stem cells are self-renewing multipotent cells within tumours that initiate tumour growth and have metastatic potential[53]. Although the normal gastric stem cell has yet to be definitively identified, it has been presumed that in the corpus of the stomach, it is located in the isthmus of the gastric glands from which it gives rise to differentiated daughter cells via bidirectional migration patterns[54]. In the antrum of the stomach, the stem cells are believed to be located at the bottom of the glands from which daughter cells migrate towards the surface unidirectionally[55]. Another possible source for gastric cancer stem cells from outside the stomach is the bone marrow. Bone-marrow-derived cells (BMDC) were elegantly shown in *H. pylori*-infected mice to repopulate the stomach before progressing through metaplasia and dysplasia to intra-epithelial cancer[56].

CLINICAL APPLICATIONS

The application of research findings to patient care is commonly found in the areas of molecular

diagnosis, prognostication and therapeutics. For example, the differential expression of *c-erb*B-2 and K-*sam* has led to the possibility of using them as prognostic markers for intestinal and diffuse type cancer respectively[35]. VEGF-positive tumours have also been shown to have a worse prognosis than VEGF-negative tumours[35]. *CDH1* and E-cadherin are already in use as screening tools for HDGC and the diagnostic and prognostic strength of the association with diffuse cancer is sufficient to propose "prophylactic" total gastrectomy for affected family members[57]. The resected stomachs almost invariably show microscopic intramucosal signet cell cancer not detected by conventional means[58]. Target-protein-based cancer therapy is also possible using monoclonal antibodies, small molecules or anti-sense oligonucleotides for inhibition of specific proliferative factors[59]. Agents like imatinib and trastuzumab have been developed for chronic myeloid leukaemia and breast cancer, and although no specific drug has yet to be developed for gastric cancer, some of the existing agents have shown promise in the treatment of advanced disease. Trastuzumab (Herceptin) is a monoclonal antibody that binds to the HER2 transmembrane receptor tyrosine kinase protein thus inhibiting cellular proliferation. It has been shown to have a statistically significant effect on prolonging overall survival (13.5 months vs 11.1 months at 17.1 months follow-up) in a recent trial for the treatment of advanced gastric cancer (ToGa)[60–62]. A clinical trial involving trastuzumab in combination with TS-1 and cisplatin in advanced gastric cancer patients is currently ongoing in the National Cancer Centre of Singapore. Other agents that have been investigated include bevacizumab (Avastin) and borteozomib (Velcade) although neither has shown as much promise as trastuzumab. Preliminary results of the AVAGAST Phase III trial suggested that bevacizumab had no beneficial impact on the overall survival of metastatic gastric cancer patients when added to Xeloda and cisplatin chemotherapy.

CONCLUSION

The ongoing research on the molecular aspects of gastric cancer has yielded a wealth of knowledge that is finally beginning to have clinical applications in the diagnosis, prognostication and therapy of this lethal disease. Although our understanding of the disease is still far from comprehensive, further work in the exciting area of cancer stem cells and cancer pathways is likely to provide us with novel methods to detect and treat gastric carcinoma in future.

REFERENCES

1. Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. Methods Mol Biol. 2009;472:467–77.
2. National Registry Diseases Office. Singapore Cancer Registry Interim Report: Trends in Cancer Incidence in Singapore 2002–2006 [Internet]. Singapore: Singapore Cancer Registry and Health Promotion Board, Singapore; 2008 [cited 2010 Apr 1]. [about 15 p.] Available from: http://www.hpb.gov.sg/uploadedFiles/HPB_Online/Publications/CancerTrends2002-2006.pdf.
3. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009 Jul–Aug;59(4):225–249.
4. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49.
5. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988 Jul 1;48(13):3554–60.
6. Milne AN, Carneiro F, O'Morain C, Offerhaus GJA. Nature meets nurture: molecular genetics of gastric cancer. Hum Genet. 2009 Nov;126(5):615–28. Epub 2009 Aug 6.
7. Chan AO, Luk JM, Hui WM, Lam SK. Molecular biology of gastric carcinoma: from laboratory to bedside. J. Gastroenterol Hepatol. 1999 Dec;14(12):1150–60.
8. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002 Dec 19;420(6917):860–67.
9. Hamilton JP, Meltzer SJ. A review of the genomics of gastric cancer. Clin Gastroenterol Hepatol 2006 Apr;4(4):416–25.
10. Nardone G, Rocco A, Malfertheiner P. Review article: helicobacter pylori and molecular events in precancerous gastric lesions. Aliment Pharmacol Ther. 2004 Aug 1;20(3):261–70.
11. Woolf CM, Isaacson EA. An analysis of 5 "stomach cancer families" in the state of Utah. Cancer. 1961 Oct;14:1005–16.
12. Werner M, Becker KF, Keller G, Höfler H. Gastric adenocarcinoma: pathomorphology and molecular pathology. J Cancer Res Clin Oncol. 2001 Mar 4; 127(4):207–16.
13. Hohenberger P, Gretschel S. Gastric cancer. Lancet. 2003 Jul 26;362(9380):305–15.
14. Smith M, Hold G, Tahara E, El-Omar E. Cellular and molecular aspects of gastric cancer. World J Gastroenterol. 2006 May 21;12(19):2979–90.
15. Egan BJ, Holmes K, O'Connor HJ, O'Morain CA. Helicobacter pylori gastritis, the unifying concept for gastric diseases. Helicobacter. 2007 Nov;12 Suppl 2:39–44.
16. Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am. 2000 Sep;29(3):559–78.
17. Keates S, Keates AC, Warny M, Peek RM, Murray PG, Kelly CP. Differential activation of mitogen-activated protein kinases in AGS gastric epithelial cells by cag+ and cag- Helicobacter pylori. J Immunol. 1999 Nov 15;163(10):5552–59.
18. Peek RM, Crabtree JE. Helicobacter infection and gastric neoplasia. J Pathol. 2006;208(2):233–48.
19. El-Omar EM, Carrington M, Chow W, McColl KEL, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000 Mar 23;404(6776):398–402.
20. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardia gastric cancer associated with proinflammatory

cytokine gene polymorphisms. Gastroenterology. 2003 May;124(5):1193–201.
21. Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, et al. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Biomarkers Prev. 2005 Nov; 14(11):2487–93.
22. Grigorova M, Staines JM, Ozdag H, Caldas C, Edwards PAW. Possible causes of chromosome instability: comparison of chromosomal abnormalities in cancer cell lines with mutations in BRCA1, BRCA2, CHK2 and BUB1. Cytogenet Genome Res. 2004;104(1–4):333–40.
23. Lee M, Tseng R, Hsu H, Chen J, Tzao C, Ho WL, et al. Epigenetic inactivation of the chromosomal stability control genes BRCA1, BRCA2, and XRCC5 in non–small cell lung cancer. Clinl Cancer Res. 2007 Feb 1;13(3):832–8.
24. Moss SF. Review article: Cellular markers in the gastric precancerous process. Aliment Pharmacol Ther. 1998 Feb;12 Suppl 1:91–109.
25. 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 Mar;187(4):428–32.
26. Lee J, Park S, Abraham SC, Seo J, Nam J, Choi C, et al. Frequent CpG island methylation in precursor lesions and early gastric adenocarcinomas. Oncogene. 2004 Apr 5;23(26):4646–54.
27. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003 Nov 20;349(21):2042–54.
28. Zheng L, Wang L, Ajani J, Xie K. Molecular basis of gastric cancer development and progression. Gastric Cancer. 2004;7(2):61–77.
29. Yokota J, Yamamoto T, Miyajima N, Toyoshima K, Nomura N, Sakamoto H, et al. Genetic alterations of the c-erbB-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-erbA homologue. Oncogene. 1988 Mar;2(3):283–7.
30. Vizoso FJ, Corte MD, Alvarez A, García I, del Casar JM, Bongera M, et al. Membranous levels of c-erbB-2 oncoprotein in gastric cancer: their relationship with clinicopathological parameters and their prognostic significance. Int J Biol Markers. 2004 Dec;19(4):268–74.
31. Yonemura Y, Ninomiya I, Ohoyama S, Kimura H, Yamaguchi A, Fushida S, et al. Expression of c-erbB-2 oncoprotein in gastric carcinoma. Immunoreactivity for c-erbB-2 protein is an independent indicator of poor short-term prognosis in patients with gastric carcinoma. Cancer. 1991 Jun 1;67(11):2914–18.
32. Katoh M, Hattori Y, Sasaki H, Tanaka M, Sugano K, Yazaki Y, et al. K-sam gene encodes secreted as well as transmembrane receptor tyrosine kinase. Proc Natl Acad Sci USA. 1992 Apr 1;89(7):2960–64.
33. Terada M, Sakamoto H, Ohmura Y, Tsuruta H, Akiyama N, Sasaki H, et al. Biological significance of gene amplification in carcinogenesis. Princess Takamatsu Symp. 1991;22:371–80.
34. Hattori Y, Itoh H, Uchino S, Hosokawa K, Ochiai A, Ino Y, et al. Immunohistochemical detection of K-sam protein in stomach cancer. Clin Cancer Res. 1996 Aug;2(8):1373–81.
35. Becker K, Keller G, Hoefler H. The use of molecular biology in diagnosis and prognosis of gastric cancer. Surg Oncol. 2000 Jul;9(1):5–11.
36. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Ito H, Tahara E. Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun. 1992 Nov 30;189(1):227–32.
37. Shiao YH, Rugge M, Correa P, Lehmann HP, Scheer WD. p53 alteration in gastric precancerous lesions. Am J Pathol. 1994 Mar;144(3):511–17.
38. Joypaul BV, Newman EL, Hopwood D, Grant A, Qureshi S, Lane DP, et al. Expression of p53 protein in normal, dysplastic, and malignant gastric mucosa: an immunohistochemical study. J Pathol. 1993 Jul;170(3):279–83.
39. Tahara E. Genetic alterations in human gastrointestinal cancers. The application to molecular diagnosis. Cancer. 1995 Mar 15;75(6 Suppl):1410–17.
40. Subramaniam MM, Chan JY, Yeoh KG, Quek T, Ito K, Salto-Tellez M. Molecular pathology of RUNX3 in human carcinogenesis. Biochim Biophys Acta. 2009 Dec;1796(2):315–31. Epub 2009 Aug 12.
41. Fukamachi H, Ito K. Growth regulation of gastric epithelial cells by Runx3. Oncogene. 2004 May 24;23(24):4330–5.
42. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, et al. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res. 1994 Jul 15;54(14):3845–52.
43. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol. 2006 Jan;14(1):21–33.
44. Pharoah PD, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology. 2001 Dec;121(6):1348–53.
45. Oliveira C, Sousa S, Pinheiro H, Karam R, Bordeira-Carriço R, Senz J, et al. Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology. 2009 Jun;136(7):2137–48.
46. Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. Cell. 1982 May;29(1):245–55.
47. Blackburn EH. Structure and function of telomeres. Nature. 1991 Apr 18;350(6319):569–73.
48. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in tetrahymena extracts. Cell. 1985 Dec;43(2, Part 1):405–13.
49. Yasui W, Tahara H, Tahara E, Fujimoto J, Nakayama J, Ishikawa F, et al. Expression of telomerase catalytic component, telomerase reverse transcriptase, in human gastric carcinomas. Jpn J Cancer Res. 1998 Nov;89(11):1099–103.
50. Xie K, Huang S. Regulation of cancer metastasis by stress pathways. Clin Exp Metastasis. 2003;20(1):31–43.
51. Lechner M, Rieder J, Tilg H. Helicobacter pylori infection, iNOS, and gastric cancer: the impact of another possible link. J Surg Oncol. 2007 Mar 1;95(3):271–72.
52. Chen C, Hsieh F, Cheng Y, Chang K, Lee P. Expression of inducible nitric oxide synthase and cyclooxygenase-2 in angiogenesis and clinical outcome of human gastric cancer. J Surg Oncol. 2006 Sep 1;94(3):226–33.
53. Karam SM. Cellular origin of gastric cancer. Ann N Y Acad Sci. 2008 Sep;1138:162–8.
54. Quante M, Wang TC. Stem cells in gastroenterology and hepatology. Nat Rev Gastroenterol Hepatol. 2009 Dec;6(12):724–37.
55. Takaishi S, Okumura T, Wang TC. Gastric cancer stem cells. J Clin Oncol. 2008 Jun 10;26(17):2876–82.
56. Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, et al. Gastric cancer originating from bone marrow-derived cells. Science. 2004 Nov 26;306(5701):1568–71.
57. Ziogas D, Roukos DH. CDH1 Testing: can it predict the prophylactic or therapeutic nature of total gastrectomy in hereditary diffuse gastric cancer? Ann Surg Oncol. 2009 Oct;16(10):2678–81.
58. Mayrbaeurl B, Keller G, Schauer W, Burgstaller S, Czompo M, Hoebling W, et al. Germline mutation of the E-cadherin gene in three sibling cases with advanced gastric cancer: clinical consequences for the other family members. Eur J Gastroenterol Hepatol. 2010 Mar;22(3):306–10.

59. Wu K, Nie Y, Guo C, Chen Y, Ding J, Fan D. Molecular basis of therapeutic approaches to gastric cancer. J Gastroenterol Hepatol. 2009 Jan;24(1):37-41.
60. Rebischung C, Barnoud R, Stéfani L, Faucheron J, Mousseau M. The effectiveness of trastuzumab (Herceptin) combined with chemotherapy for gastric carcinoma with overexpression of the c-erbB-2 protein. Gastric Cancer. 2005;8(4):249–52.
61. Jørgensen JT. Targeted HER2 treatment in advanced gastric cancer. Oncology. 2010 Feb 25;78(1):26–33.
62. Roukos DH. Targeting gastric cancer with trastuzumab: new clinical practice and innovative developments to overcome resistance. Ann Surg Oncol. 2010 Jan;17(1):14–7.