2

Early Onset Gastric Cancer: On the road to unraveling gastric carcinogenesis

AN Milne[1,2], R Sitarz[2,3], R Carvalho[2], F Carneiro[4], GJA Offerhaus[1,2]

1 Department of Pathology, University Medical Centre, Utrecht, The Netherlands
2 Department of Pathology, Academic Medical Centre, Amsterdam, The Netherlands
3 Department of Human Anatomy, Medical University of Lublin, Poland
4 Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP) and Medical Faculty/Hospital S. João, Porto, Portugal

Current Molecular Medicine 2007 Feb;7(1):15-28.

Abstract

Gastric cancer is thought to result from a combination of environmental factors and the accumulation of specific genetic alterations due to increasing genetic instability, and consequently affects mainly older patients. Less than 10% of patients present with the disease before 45 years of age (early onset gastric carcinoma) and these patients are believed to develop gastric carcinomas with a molecular genetic profile differing from that of sporadic carcinomas occurring at a later age. In young patients, the role of genetics is presumably greater than in older patients, with less of an impact from environmental carcinogens. As a result, hereditary gastric cancers and early onset gastric cancers can provide vital information about molecular genetic pathways in sporadic cancers and may aid in the unraveling of gastric carcinogenesis.

This review focuses on the molecular genetics of gastric cancer and also focuses on early onset gastric cancers as well as familial gastric cancers such as hereditary diffuse gastric cancer. An overview of the various pathways of importance in gastric cancer, as discovered through *in-vitro*, primary cancer and mouse model studies, is presented and the clinical importance of *CDH1* mutations is discussed.

Background

Gastric cancer is the fourth most common malignancy in the world and ranks second in terms of cancer-related death.[1] Eastern Asia, the Andean regions of South America and Eastern Europe have the highest incidence of gastric cancer whereas low rates are found in North America, Northern Europe and most countries in South eastern Asia.

Several classification systems have been proposed, but the most commonly used are those of the World Health Organization (WHO) and of Laurén who describes two main histological types, diffuse and intestinal.[2] Intestinal adenocarcinoma predominates in the high-risk areas whereas the diffuse adenocarcinoma is more common in low-risk areas.[3] Although classification varies between Japan and the West, attempts have been made recently to standardize the systems used.[4] Early gastric cancer is a term to describe carcinomas limited to the mucosa or to both the mucosa and submucosa, regardless of nodal status. The prevalence of this lesion is higher in countries such as Japan where a screening programme is carried out.

Gastric cancer is thought to result from a combination of environmental factors and the accumulation of generalized and specific genetic alterations, and consequently affects mainly older patients often after a long period of atrophic gastritis. The commonest cause of gastritis is infection by *Helicobacter Pylori*, which is the single most common cause of gastric cancer[5, 6] and has been classified by the WHO as a class I carcinogen since 1994.[7] The risk of infection varies with age, geographical location and ethnicity, but overall 15-20% of infected patients develop gastric or duodenal ulcer disease and less than 1% will develop gastric adenocarcinoma. [7]

The pattern of gastritis has also been shown to correlate strongly with the risk of gastric adenocarcinoma. The presence of antral-predominant gastritis, the most common form, confers a higher risk of developing peptic ulcers; whereas corpus predominant gastritis and multifocal atrophic gastritis leads to a higher risk of developing gastric ulcers and subsequent gastric cancer.[8, 9] The response to *Helicobacter Pylori* infection and the subsequent pattern of gastritis depends on the genotype of the patients and in particular a polymorphism in interleukin 1 beta, an inflammatory mediator triggered by *Helicobacter Pylori* infection, is known to be of importance.[10] Multifocal atrophic gastritis is usually accompanied by intestinal metaplasia and leads to cancer via dysplasia, and thus intestinal metaplasia is considered a dependable morphological marker for gastric cancer risk. Unlike intestinal gastric cancer, the diffuse type typically develops following chronic inflammation without passing through the intermediate steps of atrophic gastritis or intestinal metaplasia.

The incidence of adenocarcinoma of the stomach is declining worldwide and this is mainly accounted for by the decline in the intestinal type. There has also been a change in the anatomical distribution of this malignancy over recent decades, with a fall in the incidence of mid and distal gastric cancer and a progressive increase in adenocarcinoma of the proximal stomach and cardia. This fall in incidence may be explained by the decline in *Helicobacter pylori* infection and associated atrophic gastritis. The possibility that the increasing incidence of adenocarcinoma of the cardia may be due to nitrosative chemistry is discussed by McColl *et al.*[11]

The exact mechanism underlying the malignant transformation of the gastric mucosa following *Helicobacter pylori* infection still needs to be clarified, but it is believed that the combination of a virulent organism, a permissive environment and a genetically susceptible host is necessary.[12, 13] Different strains of the bacteria vary in their carcinogenic potential, with those containing cag genes inducing a greater degree of inflammation. *Helicobacter pylori* can also produce the vacuolating cytotoxin VacA responsible for epithelial damage which contributes to gastric carcinogenesis.

Bacterial factors (motility, adhesion, urease, cag pathogenicity), components of the host immune response (Toll-like receptors, adaptive immunity, IL1-B polymorphisms, MHCII), dietary co-factors such as high salt and decreased ascorbic acid, gastrin hormonal responses and decreased acid secretion are all thought to play a role in malignant transformation of the gastric mucosa.[14] In addition, IL-8, heat shock proteins and proinflammatory cytokines, nitric oxide and oxidative stress have also been implicated in gastric carcinogenesis. All these factors interact to alter host cell signaling, derange apoptotic and proliferative signaling and promote the accumulation of genetic alterations leading ultimately to neoplasia as reviewed by Stoicov *et al*.[14] Interestingly, despite the importance of *Helicobacter pylori* as an initiating factor in gastric carcinogenesis, the molecular pathology of *Helicobacter pylori* and non-*Helicobacter pylori* cancers cannot be easily separated, and it has been reported that *Helicobacter pylori* -related and non-related gastric cancers do not differ with respect to chromosomal aberrations.[15]

Diet is also a known etiological factor in gastric carcinogenesis, especially for intestinal type adenocarcinoma. An adequate intake of fruit and vegetables appears to lower the risk with ascorbic acid, carotenoids, folates and tocopherols acting as antioxidants.[3] Salt intake strongly associates with the risk of gastric carcinoma and its precursor lesions, and this risk is increased in certain genetically predisposed individuals.[16] Other foods associated with high risk in some populations, include smoked or cured meats and fish, pickled vegetables and chilli peppers.[3] Alcohol, tobacco and occupational exposure to nitrosamines and inorganic dusts have been studied in several populations, but the results have been inconsistent.[3]

Epstein-Barr virus (EBV) which is observed in 7%-20% of gastric cancers and which occurs slightly more frequently in diffuse-type gastric cancers, has also been implicated in gastric carcinogenesis.[17] In addition, it is known that a Bilroth II operation, which leaves a remnant or gastric stump, increases the risk of gastric carcinoma more than 15 years after surgery, [18] possibly due to bile reflux.

Curative therapy of gastric cancer involves surgical resection (discussed in a review by Ushijima *et al* [19]), and most commonly takes the form of a total or subtotal gastrectomy, with an accompanying lymphadenectomy. However, substantial mortality associated with gastric cancer has prevailed despite technical advances in surgery and adjuvant therapy, and the overall 5-year survival rate in patients with resectable gastric cancer remains between 10% and 30%. Furthermore, the lack of early pathognomic symptoms often delays the diagnosis and although endoscopy is widely regarded as the most sensitive and specific diagnostic test for gastric cancer, infiltration of the gastric wall, cannot always be detected. Clinical features, diagnosis and treatment of gastric cancer are reviewed comprehensively by Dicken *et al*. [20]

Gastric cancer can be categorized into conventional gastric cancer, occurring in patients older than 45, early-onset gastric cancer (EOGC), occurring under 45 years old and gastric cancer occurring as part of a hereditary syndrome. This review will first deal with the molecular pathology of gastric cancer in the broad sense before focusing on the findings specific to EOGC and hereditary gastric cancer and how they can be used to examine gastric cancer as a whole.

Molecular Pathology of Gastric Cancer

Tumorigenesis is considered a multistep process involving generalized and specific genetic alterations that drive the progressive transformation of cells into cancer. Central to this transformation are genetic or epigenetic changes in the genome which specifically activate

oncogenes with a dominant gain of function, and produce alterations in tumor suppressor genes which cause loss of function. Hanahan and Weinberg [21] describe in a compelling review how virtually all mammalian cells carry a similar molecular machinery regulating their proliferation, differentiation, and death and suggest that there are six essential alterations in cell physiology that collectively dictate malignant growth. These comprise self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. They outline that each of these capacities acquired during tumor development represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues. In addition, they mention genetic instability as a precondition for tumorigenesis through disruption of key molecules in order to "fast-forward" their carcinogenic potential. This framework described by Hanahan and Weinberg [21] can be applied to gastric cancer to highlight the important advances in molecular knowledge in the field of gastric cancer through *in-vitro*, primary tumors and mouse model experiments. It is however important to bear in mind that in practice, many molecular functions can play a role in a number of these six critical processes, and certain molecules disrupted in cancer have wide-ranging functions.

Self-sufficiency in growth signals and oncogenes

The dependence of tumors on communication from neighboring cells can be relinquished by the autonomous production of growth factors, which in turn results in the disruption of critical homeostatic mechanisms. In this manner, alterations in growth factor receptors, integrins and downstream signaling pathways serve as oncogenes, driving the carcinogenic process.

In gastric cancer there have been a number of oncogenes implicated. K-sam, which belongs to the family of fibroblast growth factor receptors (FGFR) is frequently overexpressed in diffuse-type gastric cancers due to gene amplification.[22, 23] Growth factors of the epidermal growth factor (EGF) family and their respective receptors including c-erbB2 oncogene are preferentially overexpressed in intestinal gastric cancers.[24, 25] In addition, the c-met proto-oncogene which is the receptor for the hepatocyte growth factor (HGF) is frequently overexpressed in gastric cancers of both diffuse and intestinal type. [22, 26]

Interestingly, many oncogenes which are key players in other epithelial cancers do not play a central role in gastric cancer. For example, Ras proteins are present in structurally altered oncogenic forms in about 25% of human tumors. Despite a mutant *K-ras* oncogene mouse model which showed pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia, [27] mutation of this oncogene occurs very rarely in gastric cancer. Similarly, the role of the Wnt pathway which is central to colorectal carcinogenesis, remains unclear in gastric cancer. Activating mutations of β-catenin have been described in gastric cancer [28] and immunohistochemical abnormalities are present in 22-27% of gastric cancer [29, 30]; yet as outlined later, the importance of *APC* mutations in gastric cancer is not yet fully understood. Of note, the transcription factor c-myc which is a transcriptional target of many pathways including the Wnt signalling pathway, functions as an oncogene in gastric cancer, with overexpression causing impaired differentiation and promoting growth.[31] Overexpression of c-myc has been described in over 40% of gastric cancers.[30]

Proliferation of the gastric mucosa is regulated by numerous different mechanisms, one of which is endocrine regulation via the hormone gastrin. *Helicobacter* infection induces hypergastrinemia and this has been causally linked to increased proliferation and cancer. Infection in the insulin-gastrin transgenic mouse produces an early increase in acid secretion and over time progresses to

atrophy, achlorhydria, hyperplasia of mucous cell compartment, metaplasia, dysplasia and invasive gastric cancer by 8 months of age.[32] Conversely, gastrin deficiency has also been reported to cause gastric adenocarcinoma.[33] In addition, *Helicobacter pylori* infection also alters gastric mucosal signaling through the CagA protein which interacts with several major growth-regulating signal transduction pathways including the Ras/MEK/ERK pathway[34] and the Src family of protein kinases.[35]

Intestinal homeostasis is disrupted in tumor cells through numerous mechanisms. COX-2, one of the rate-limiting enzymes for prostaglandin synthesis from arachidonic acid, is frequently upregulated in gastric adenocarcinomas and its expression is thought to be a relatively early event in gastric carcinogenesis.[36] In fact *Helicobacter pylori* infection has been reported to induce overexpression of COX-2.[37, 38] The role of COX-2 in gastric carcinogenesis is reviewed by Saukkonen *et al*.[39] Recently, the molecule C/EBP-β, a transcription factor for COX-2, [40] has been shown to play a role in gastric cancer.[30, 41]

Intestinal homeostasis is maintained under normal circumstances by molecules such as mucin core proteins (MUC), the expression of which has been found to vary in the different types of intestinal metaplasia.[42] In addition, due to the recent attention given to the activation and silencing of developmental pathways in cancer initiation and progression, focus has been drawn to the *Drosophila caudal*-related homeobox transcription factors Cdx1 and 2 which are important for early differentiation and maintenance of intestinal epithelial cells. Notably, ectopically-expressed Cdx2 was found to induce gastric intestinal metaplasia in two separate mouse models.[43, 44] However, progression to dysplasia and cancer occurred in only one of these models and the neoplastic role of Cdx2 remains speculative. Interestingly, both Cdx1 and Cdx2 have been shown to be expressed in intestinal metaplasia and gastric carcinomas in the human stomach.[45]

Insensitivity to growth-inhibitory signals

Cancer cells must evade antiproliferative signals if they are to survive, and the inactivation of tumor suppressor genes is a common event in gastric carcinogenesis. This can occur through mutations, deletions and epigenetic events. Methylation is an epigenetic process causing chromatin structure modulation, transcriptional repression and the suppression of transposable elements, and so is functionally equivalent to alterations such as mutations and deletions. However, a major difference is that epigenetic inactivation can be abrogated by DNA methylation inhibitors, and may be reversible. Hypermethylation in gastric cancer is extensively reviewed by Sato *et al*.[46] A genome-wide scan for aberrant methylation revealed silencing of nine genes in gastric cancers[47] and even in non-cancerous gastric mucosa, aberrant methylation can be present.[48] Of note, nitric oxide has also been shown to induce methylation. [49]

As outlined below, a vast array of tumor suppressor genes have been implicated in gastric cancer including *TP53*, *p16*, *APC*, *TGF*-β and related molecules, *TFF1*, *SOCS1*, *testin*, *FHIT* and *RUNX3*. On the other hand, tumor suppressor genes such as *PTEN*, despite playing a vital role in many carcinomas, do not have an important role in gastric carcinogenesis.[50]

The tumor suppressor gene *TP53* encodes for a nuclear protein, which plays a key role in tumor progression by regulating DNA repair, cell division and apoptosis. Low apoptosis rate and high cell proliferation are thought to be important factors for gastric cancer development and inactivation of p53 may be central to gastric carcinogenesis. Mutation and/or LOH at the *TP53* locus has been reported in approximately 30-40% of gastric cancers, but can also be found in intestinal metaplasia.[51]

The important cell cycle regulator, p16 (transcribed from *CDKN2A*) is lost in many gastric cancers, particularly cardia tumors,[30] and methylation has been shown to be of importance in the downregulation of this gene.[52] Additionally, EBV-associated gastric cancers have been shown to be more frequently associated with promoter methylation of *CDKN2A*. [53]

Adenomatous polyposis coli (*APC*) is a tumor suppressor gene which is mutated in sporadic and familial colorectal tumors. Under normal circumstances, APC binds to β-catenin and induces its degradation. Mutations of APC or β-catenin result in stabilization and accumulation of β-catenin, which can then translocate to the nucleus, where it acts as an oncoprotein, through transcription of target genes. This is a well-established mechanism in colorectal cancer, however less is known about the relative importance of this pathway in gastric cancer. Whereas some reports document relatively frequent occurrence of mutations,[54, 55] others find no mutations.[56, 57] The complexity is further increased by a report finding an inverse relationship between *APC* gene mutation in gastric adenomas and the development of adenocarcinoma.[58] Interstingly, *CDH1* and *APC* mutations have been reported to be synergistic in intestinal tumor initiation in mice[59] whereby double heterozygous animals showed a significant 5-fold increase in gastric tumor numbers, compared with Apc1638N animals.

Another feature in gastric carcinogenesis is the loss of growth inhibition by transforming growth factor (TGF)-β due to mutation of the Type II TGF-β receptor,[60] which leads to increased cell proliferation and reduced apoptosis. In addition, the cytoplasmic Smad4 protein, which transduces signals from ligand- activated TGFβ receptors to downstream targets, may be eliminated through mutation of its encoding gene. Loss of the locus encompassing *SMAD4* (18q21.1) and *DCC*(18q21.3) locus has been long known, [61] but more recently, haploid loss of this locus has been shown to initiate gastric polyposis and cancer in *Smad4+/-* mice.[62] Loss of the remaining *Smad4* wild-type allele was detected only in later stages of tumor progression, suggesting that haplo-insufficiency of Smad4 is sufficient for tumor initiation. Furthermore, bone morphogenetic protein (BMP)-2, a member of the BMP family belonging to the TGF-β superfamily has been shown to inhibit cell growth, and induced cell differentiation in normal and cancerous gastric cell lines.[63] Epigenetic silencing of the *BMP2* through methylation in gastric carcinomas has recently been described and noted to occur more frequently in diffuse type than intestinal type gastric cancers.[64]

Trefoil factor 1 (TFF1, also known as pS2) is synthesized and secreted by the normal stomach mucosa and by the gastrointestinal cells of injured tissues. The link between mouse Tff1 inactivation and the fully penetrant antropyloric tumor phenotype[65] prompted the classification of *TFF1* as a gastric tumor suppressor gene. Accordingly, altered expression, deletion, and/or mutations of the *TFF1* gene have been observed in human gastric carcinomas.[30, 66] The *Tff1* knock-out mice were subsequently shown to have overexpression of Cox-2 [67] and this inverse link between TFF1 and COX-2 has been confirmed in other studies.[30] TFF1 expression is in part regulated by interleukin-6 (IL-6), but the downstream intracellular signaling mechanisms of the IL-6 family of cytokines are not well understood. Mouse models have been used in an attempt to elucidate the function of the signal transducers and activators of transcription 1 and 3 (STAT1/3) and the Src-homology tyrosine phosphatase 2 (SHP2)-Ras-ERK, which are the two major signaling pathways emanating from gp130, the IL-6 family co-receptor in the gastrointestinal tract. [68] Gp130(757F) mice, with a 'knock-in' mutation abrogating SHP2-Ras-ERK signaling, developed gastric adenomas by three months of age. In contrast, mice harboring the reciprocal mutation ablating STAT1/3 signaling, or deficient in IL-6-mediated gp130 signaling showed impaired colonic mucosal wound healing. These gastrointestinal phenotypes are highly similar to

the phenotypes exhibited by mice deficient in trefoil factor 1 (pS2/TFF1) and intestinal trefoil factor (ITF)/TFF3 respectively. In further studies, mice lacking the SHP2 binding site on the gp130 were found to develop invasive gastric cancer by 30 weeks of age, [69] highlighting the need for balanced IL-6 signaling in maintaining gastric homeostasis. More recently, a gp130 mutant mouse model with exaggerated Stat3 activation [70] was found to share histological features of gastric polyps in ageing mice with monoallelic null mutations in Smad4 and the investigators suggest a novel link for cross-talk between STAT and SMAD signaling in gastric homeostasis. Downstream, the phosphorylated STAT protein translocates into the nucleus with subsequent activation of target genes. One of the STAT-activated genes, suppressor of cytokine signalling-1 (*SOCS-1*), is thought to be an important tumor suppressor gene in gastric cancer and can be inactivated though hypermethylation.[71, 72]

Since 1996, *FHIT*, a fragile locus exhibiting susceptibility to carcinogen-induced alterations, has been implicated in gastric carcinogenesis. [73] The consequent absence or reduction of FHIT protein expression is consistent with the proposal that the *FHIT* gene is a preferential target for environmental carcinogens and this may also account for the geographical differences found in *FHIT* aberrations.[74] More recent data showed that *FHIT* knock-out mice [75] develop tumors in the lymphoid tissue, liver, uterus, testis, fore-stomach and small intestine, together with structural abnormalities in the small intestinal mucosa suggesting that *FHIT* plays important roles in systemic tumor suppression and in the integrity of mucosal structure of the intestines. In another recent knock-out mouse model a tumor suppressor function for Testin was proposed [76] and it was suggested that *TES* may be a one-hit TS gene, as is *FHIT*.[77]

RUNX3 is another gene which has been hotly debated regarding its possible tumor suppressor function in gastric carcinogenesis. The debate arises due to the conflicting mouse models reported in the literature [78, 79] which are discussed by Levanon et al.[80] More recently, it has been found that *RUNX3* can be overexpressed in gastric tumors and that copy numbers of the *RUNX3* locus are seldom reduced in gastric cancer.[81]

Finally, insensitivity to growth-inhibitory signals can also be facilitated by *Helicobacter pylori* infection and it has been found that *Helicobacter pylori* decreases levels of the cyclin-dependent kinase inhibitor p27(kip1) in gastric epithelial cell, [82] which results in a decrease in apoptotic response to infection.[83] In addition, a recent mouse model lacking p27kip1 demonstrated that loss of p27 and *Helicobacter pylori* colonization cooperate to produce gastric cancer.[84]

Apoptosis

Acquired resistance toward apoptosis is a hallmark of most and perhaps all types of cancer.[21] Many of the signals that elicit apoptosis converge on the mitochondria, which respond to proapoptotic signals by releasing cytochrome C, a potent catalyst of apoptosis. Members of the Bcl-2 family of proteins, which are either proapoptotic (Bax, Bak, Bid, Bim) or antiapoptotic (Bcl-2, Bcl-XL, Bcl-W) govern mitochondrial death signaling through cytochrome C release and some of these proteins have been implicated in gastric cancer.[85] In addition, p53 can elicit apoptosis by upregulating expression of proapoptotic Bax in response to DNA damage. In fact, mutation of p53 results in the removal of a key component of the DNA damage sensor which can induce the apoptotic cascade. The ultimate effectors of apoptosis include an array of intracellular proteases termed caspases. Two "gatekeeper" caspases, −8 and −9, are activated by death receptors such as FAS or by the cytochrome C released from mitochondria respectively, and the Fas Ag pathway of apoptosis is recognized as the leading cause of tissue destruction during *Helicobacter pylori* infection. Early in infection, Fas antigen-mediated apoptosis depletes parietal and chief cell

populations, leading to architectural distortion. As infection progresses, metaplastic and dysplastic glands appear, which are resistant to Fas-mediated apoptosis. Fas antigen-deficient (lpr) mice infected with helicobacter, develop gastric cancer as early as 7 months after infection.[86] Nitric oxide, while usually discussed in the context of DNA damage and mutagenesis, can also directly influence mitochondrial pathways of apoptosis[87] and also potentially plays a role in multiple levels of cell signal transduction during *Helicobacter pylori* infection. Furthermore, bacterial factors may also directly induce apoptosis.[88]

Limitless replicative potential and telomeres

Growth signal autonomy, insensitivity to antigrowth signals, and resistance to apoptosis all lead to an uncoupling of a cell's growth program from signals in its environment.[21] Evolving premalignant cell populations also acquire unlimited replicative potential during tumor progression, and this is often through telomere maintenance. Telomeres are located at the ends of chromosomes and are responsible for the maintenance of chromosomal integrity. During cell division, these telomeres become shortened. However, in transformed cells, shortening of the telomeres is inhibited by reactivation of telomerases, preventing these cells from undergoing physiological senescence. Telomere maintenance is evident in virtually all types of malignant cells usually via upregulating expression of the telomerase enzyme resulting in unlimited multiplication of cells. There is a vast array of molecules involved in telomere maintenance and in gastric cancer expression of Protection of Telomeres-1 (POT1) is associated with telomere length and correlates with tumor stage.[89]

Angiogenesis

In order to facilitate an increase in size, tumors need to develop angiogenic ability. This is achieved by signalling through integrins and adhesion molecules on endothelial cells as well as through cell-matrix and cell-cell contacts. A large number of angiogenic factors have been identified in human malignancy, and gastric cancer is no exception. These include vascular endothelial growth factor (VEGF),(possibly induced via *Helicobacter Pylori*), [90] basic fibroblast growth factor (bFGF) and IL-8, [91] which are derived from tumor cells and participate mainly in neovascularisation within gastric cancer tissue. In addition, extracellular proteases receive signals from proangiogenic integrins, and help dictate the invasive capability of angiogenic endothelial cells. The ability to induce and sustain angiogenesis seems to be acquired in discrete steps during tumor development, via an "angiogenic switch." Tumors appear to activate this switch by changing the balance of angiogenesis inducers and inhibitors.

Tissue Invasion and Metastases

Tumor metastases are the cause of 90% of human cancer deaths. Successful invasion and metastasis requires all the attributes which are needed for initial carcinogenesis, combined with alterations in proteins involved in the tethering of cells to their surroundings in a tissue. The most widely observed alteration in cell-to-environment interactions in cancer involves E-cadherin, a homotypic cell-to-cell interaction molecule ubiquitously expressed on epithelial cells and playing a central role in gastric cancer (as discussed in detail under hereditary gastric cancer). Invading and metastasizing cancer cells travel through a range of tissue microenvironments to which they adapt by producing a changing spectrum of integrin α or β subunits on their cell surfaces. The activation of extracellular proteases and the altered binding specificities of cadherins, CAMs, and integrins are central to the acquisition of invasiveness and metastatic ability and MMP2 has been shown to be of particular

importance in gastric cancer.[92] Through comparison of gastric cancer SAGE libraries, 54 candidate GC-specific genes have been identified including melanoma inhibitory activity (MIA) and matrix metalloproteinase-10 (MMP-10), which is important in metastasis and correlated with poor prognosis.[93]

Genomic instability

Under normal circumstances, the occurrence of mutations is prevented by the maintenance of genomic integrity by an array of DNA-monitoring and repair enzymes and karyotypic order is guaranteed by checkpoints that operate at critical times in the cell's life. Yet cancers occur relatively frequently in the human population, causing some to argue that the genomes of tumor cells must acquire increased mutability in order for the process of tumor progression to reach completion in several decades time. Derangement of specific components of the genomic "caretaker" systems has been used as an explanation and the loss of function of these key players is believed to result in genome instability and the generation of mutant cells with selective advantages.[21]

A variable number of numerical or structural genetic aberrations have been reported in gastric cancer cells, including those involving changes in chromosomes and DNA copy number, but the significance of these changes and the underlying genetic changes are unknown. Loss of Heterozygosity studies and comparative genetic hybridization (CGH) analyses have identified several loci with significant allelic loss, indicating possible tumor suppressor genes important in gastric carcinoma. Common targets of loss or gain include chromosomal regions 1q, 3p, 4, 5q, 6q, 9p, 17p, 18q and 20q. [61, 94-97] It has been shown that different histopathologic features can be associated with distinct patterns of gains and losses, supporting the notion that gastric tumors evolve through distinct genetic pathways.[98] Persistent inflammation caused by *Helicobacter pylori* is also known to cause genetic instability through the generation of mutagenic substances such as reactive oxygen species [99] and reactive nitrogen species [37] which may act to directly damage the host cell DNA. *Helicobacter pylori* has also been implicated in limiting the defense against such insult by decreasing the antioxidant properties of the gastric mucosa.[100] Such a direct gastric mutagenic through oxidative DNA damage in *H. pylori* infection, has been shown in transgenic mouse models. [101]

Genetic instability at the level of microsatellite instability (MSI) occurs in many sporadic human tumors and the relation between microsatellite instability and gastric carcinoma has received considerable attention. This is due to the discovery that MSI may be found in sporadic carcinomas that are characteristic of hereditary nonpolyposis colorectal cancer (HNPCC) [102], a syndrome where germline mutations of the mismatch repair genes are present. The levels of MSI found in gastric carcinomas from both Western and Eastern populations is probably in the region of up to 15%.[103] Wu *et al*. demonstrated that the subset of sporadic gastric cancer with high frequency MSI (MSI-H) showed a distinct clinicopathologic and genetic profile from those with a low frequency (MSI-L) or microsatellite stable (MSS) genotype. [104] However, whereas the role of microsatellite instability and DNA mismatch repair gene defects in HNPCC is unquestionable and well established, the relevance of this phenomenon in gastric cancer is far from clear and currently has limited clinical value.[103] Somatic mutations of mismatch repair (MMR) genes such as *hMLH1* or *hMSH2* are extremely rare in sporadic gastric cancers, with only one mutation found, in *hMSH2*. [105] However, MSI positive tumors can still lack *hMLH1* protein expression and many studies suggest that hypermethylation of the *hMLH1* promoter region may be the principal mechanism of gene inactivation in sporadic gastric carcinomas with a high frequency

of MSI.[106, 107]. The role of microsatellite instability in gastric carcinoma is comprehensively reviewed by Hayden *et al.* [103]

As is evident from the preceding text, multiple genetic and epigenetic alterations in oncogenes, tumor-supressor genes, cell-cycle regulators, cell-adhesion molecules, DNA repair genes and genetic instability as well as telomerase activation are implicated in human stomach cancer. However, particular combinations of these alterations differ in the two histological types of gastric cancer.[98] The diffuse phenotype in gastric cancer (hereditary and sporadic) is related to reduced E-cadherin expression [108] and loss of E-cadherin is probably the fundamental defect in diffuse type gastric carcinoma, providing an explanation for the observed morphological phenotype of discohesive cells with loss of polarity and gland architecture. Recent findings with E-Cadherin, C/EBP-β, TFF1 and COX-2 expression emphasize the fact that diffuse and intestinal cancers differ at a molecular level.[30] However, the onset of carcinogensis is strongly associated with *Helicobacter pylori* infection as reviewed by Nardone *et al* [109] and indeed there is close correlation between diffuse GC and *Helicobacter pylori* infection, similar to that found with intestinal type cancer.[110] Studies have also shown decreased E-Cadherin expression in the gastric mucosa of infected individuals.[111] Therefore, even if the intestinal and diffuse type GCs are characterized by a different genetic pathway, they depend upon the same triggering factor.

In addition to the wealth of research looking at specific genes of interest in gastric cancer, gene expression array data has also revealed a vast amount of information on gastric cancer. However, putting these pieces together into a chronological narrative remains daunting, and a recent approach involving a meta-analysis of previous expression array data hints at how complicated the "gastrome" can be.[112] There is by no means a clear-cut pattern of mutations in gastric cancers, and the genetic research can often be hampered by the diversity of changes that are induced by *Helicobacter pylori* infection, diet, ageing and other environmental factors. Tumors are unquestionably riddled with genetic changes yet we are faced with an unsolvable puzzle with respect to a temporal relationship. In order to solve this problem, one approach is to investigate tumors that are less influenced by these environmental factors. Gastric cancers occurring in young patients, known as early-onset gastric cancers, provide an ideal background on which to try and uncover the initiating stages in gastric carcinogenesis. In addition hereditary cancers can often illuminate discrete mutations that can initiate the pathway of gastric carcinogenesis.

Hereditary Cancer and E-Cadherin

The existence of a familial form of gastric cancer has been known since the 1800s when multiple cases of gastric cancer were noted in the Bonaparte family.[113] Approximately 1-3% of gastric cancers arise as a result of inherited gastric cancer predisposition syndromes, one of which is hereditary diffuse gastric cancer, caused by a germline mutation in the *CDH1* gene, encoding E-Cadherin. Gastric cancer in its hereditary form can also be caused by germline mutations of the *TP53* tumor suppressor gene which occurs in the Li-Fraumeni syndrome.[114] In addition, *BRCA2* gene mutations are associated with familial aggregations of not only breast but also of stomach, ovarian, pancreatic, and prostate cancers. [115, 116] A proportion of hereditary nonpolyposis colorectal cancer (HNPCC) kindreds (the so-called Lynch II families) are associated with a high frequency of extracolonic carcinomas, most commonly affecting the endometrium and stomach [117] and these are known to harbor microsatellite instability. [118] In addition, gastric cancer occurs infrequently in polyposis syndromes such as familial adenomatomous polyposis

(FAP) [119] and Peutz-Jegers syndrome. [120, 121] The American Society for Gastrointestinal Endoscopy recommends endoscopic surveillance for high-risk individuals (history of gastric adenoma, FAP, HNPCC, Peutz-Jeghers syndrome, and Menetrier's disease) every 1 to 2 years.

Approximately 30% -40% of all hereditary diffuse gastric cancer (HDGC) families carry *CDH1* germline mutations.[122] The other 60%-70% of HDGC remain genetically unexplained and are probably caused by alterations in other genes. It has been suggested there may be a need for p53 mutation screening in families with hereditary gastric cancer lacking *CDH1* germline mutations.[123] No evidence has been found for a role of germline mutations in *SMAD4* and *Caspase-10* in these families. [123] E-cadherin is a member of the cadherin family of homophilic cell adhesion proteins that are central to the processes of development, cell differentiation, and maintenance of epithelial architecture.[124] It is the predominant cadherin family member expressed in epithelial tissue and is localised at the adherens junctions on the basolateral surface of the cell. Mutations in *CDH1* were initially identified in 1998 in three Maori families from New Zealand that were predisposed to diffuse gastric cancer. [125] Since then, similar mutations have been described in more than 40 additional HDGC families of diverse ethnic backgrounds.[122] Preliminary data from these families suggest that the penetrance of *CDH1* gene mutations is high, ranging between 70% and 80%.[126] In order to qualify for a diagnosis of HDGC, the following criteria must be met [127]: two or more documented cases of diffuse gastric cancer in first or second degree relatives, with at least one diagnosed before the age of 50 years; or three or more cases of documented diffuse gastric cancer in first or second degree relatives, independent of age of onset. Death from gastric cancer in these families has occurred in individuals as young as 14 years and the majority of affected persons die aged less than 40 years. There also appears to be an increased frequency of cancers occurring at other sites such as the breast, colorectum, and prostate in these mutation carriers.[126] However, inclusion of associated cancers into the definition of HDGC is not yet recommended.[127]

Abnormalities of CDH1

CDH1 is a tumor suppressor gene and loss or inactivation of the remaining normal allele is a required initiating event in susceptible individuals with a germline mutation. Analysis of all reported genetic abnormalities in *CDH1* found in HDGC reveals that the majority are inactivating mutations (splice site, frameshift, and nonsense) rather than missense mutations. Furthermore, *CDH1* germline mutations are evenly distributed along the E-cadherin gene, in contrast with the clustering in exons 7-9 observed in sporadic diffuse gastric cancer.[128] Loss of heterozygosity as the "second hit" does not appear to be frequent in HDGC. Instead, hypermethylation of the *CDH1* promoter is likely to be a common cause of down-regulation or inactivation of the second *CDH1* allele in HDGC tumors.[129] The verdict has not yet been reached concerning the possible carcinogenic role of coexistent infection with *Helicobacter pylori* on a *CDH1* mutated background, and it remains possible that *Helicobacter pylori* infection as well as dietary and other environmental influences may modify the disease risk in these susceptible individuals.[130]

Clinical Management

There remains some uncertainty about clinical management and disease outcome after genetic testing for *CDH1* mutations, and the psychosocial burden it poses on family members is well recognised.[127] Once a *CDH1* mutation has been identified in an asymptomatic individual, they are presented with the options of endoscopic surveillance or prophylactic gastrectomy. The aim of surveillance is of course to identify an early curable lesion but the value of endoscopy is

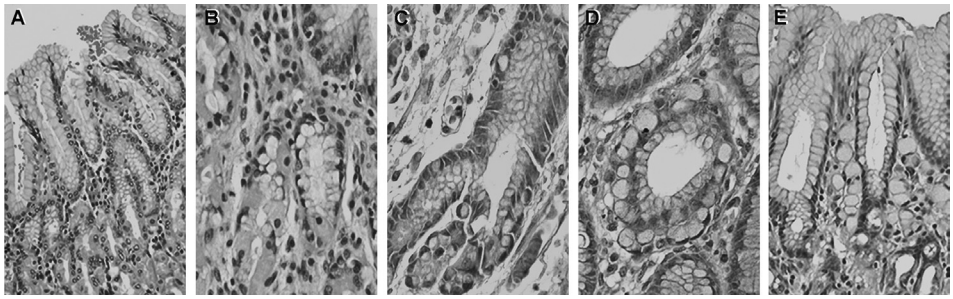

Figure 1 Proposed model for the development of diffuse gastric cancer in E-cadherin mutation carriers: background changes of gastric mucosa encompassing mild chronic gastritis and foveolar hyperplasia (A); in-situ signet-ring cell carcinoma (foveolae and glands with intact basement membrane totally or partially lined by signet ring cells) (B and C); "early"(C) and overt (D) pagetoid spread of signet-ring cells below the preserved epithelium of glands/foveolae; early invasive intramucosal signet-ring cell carcinoma (E). ***(See page 193 for colour figure)***

unproven due to the difficulty of detecting intramucosal lesions.[131] Some reports have found an antral predominance of HDGC[132] whereas other reports show no antral predominance in HDGC and alarmingly, have calculated the likelihood of detecting HDGC from five random biopsies at between 1-50%.[133] Current clinical recommendations for surveillance, propose a 30 minute endoscopy every six months by an endoscopist experienced in the diagnosis of early gastric cancer. [131] In an effort to improve the diagnostic yield of surveillance endoscopy in the upper gastrointestinal tract, techniques such as chromoendoscopy are advised.[134] In addition, all patients having surveillance should be entered into a research protocol comparing different endoscopic methods.[131] Obviously there is a great need for the development of molecular markers in the serum or in gastric brushing in order to overcome the sampling bias inherent in current random biopsy sampling methods.

Prophylactic gastrectomy is clearly a huge undertaking and not without significant psychological and clinical effects on the patient. To date, it has been demonstrated that prophylactically resected stomachs from different families all carried multifocal signet ring cancer. [135, 136] Importantly, surveillance using endoscopy (with chromoendoscopy in some cases) and multiple mucosal biopsies failed to identify intramucosal carcinoma in all of the published cases surveyed. Thus, the estimated risk reduction of gastric cancer by gastrectomy is significant. However, it also follows that since there is an estimated 70% penetrance, a universal policy of prophylactic gastrectomy would result in 30% of HDGC mutation carriers receiving an unnecessary operation. On the other hand, it is not known whether such lesions are present in all individuals with CDH1 mutations, and whether all pathologic changes would develop into clinically significant lesions. [126] The age at which genetic testing should be performed is not yet clear from the current evidence, as at least five subjects have been reported to have developed this lethal cancer before the age of 18 years. However, since the implications of the diagnosis are far reaching, some believe that genetic screening should be reserved until the patient is able to give informed consent.[131]

Model of development of HDGC

In situ carcinoma lesions have been identified in gastrectomy specimens from patients with CDH1 mutation [133, 135] whereby foveolae and glands with intact basement membrane are totally or

partially lined by signet ring cells. Some *in situ* lesions are restricted to the neck zones (Figure 1 B and C). On the basis of the findings of these studies, a model for the development of diffuse gastric cancer in E-cadherin mutation carriers was proposed, as depicted in Figure 1, and encompassing the following lesions: *in situ* signet-ring cell carcinoma (B and C), pagetoid spread of signet-ring cells below the preserved epithelium of glands/foveolae (C – "early" pagetoid spread and D – "overt" pagetoid spread), and invasive carcinoma (E – early invasive intramucosal signet-ring cell carcinoma). The discrepancy between the numerous invasive carcinoma foci and the low number of in situ carcinoma lesions suggests that invasion of the lamina propria by signet ring cells may occur without a morphologically detectable in situ carcinoma. HDGC develops in the setting of background changes of gastric mucosa encompassing mild chronic gastritis, foveolar hyperplasia (Figure 1A), tufting, globoid change and vacuolization of superficial epithelim.[133]

The gastric mucosa in *CDH1* germline mutation carriers is normal until the second *CDH1* allele is inactivated. It is postulated that this downregulation occurs in multiple cells in the gastric mucosa, accounting for the multifocal tumor lesions which develop and [133] environmental and physiological factors such as diet, carcinogen exposure, ulceration and gastritis are suggested to promote this downregulation event. The tumor then expands slowly until additional genetic events, probably in combination with an altered microenvironment, lead to clonal expansion and tumor progression. Interestingly, because the second hit does not involve somatic, irreversible, mutation of the second *CDH1* allele, but rather more frequently occurs via methylation [129], it is plausible that the early stage lesions may be reversible. Identification of patients with germline *CDH1* mutations paves the way for studies to increase our understanding of the mechanisms by which these mutations ultimately lead to sporadic cancer as well as HDGC. The genetic changes occurring after the inactivation of *CDH1* remain to be elucidated.

Early Onset Gastric Cancer

Gastric cancer is rare below the age of 30; thereafter it increases rapidly and steadily to reach the highest rates in the oldest age groups, both in males and females. The intestinal type rises faster with age than the diffuse type and is more frequent in males than in females. Early onset gastric cancer (EOGC) is defined as gastric cancer presenting at the age of 45 or younger. Approximately 10% of gastric cancer patients fall into the EOGC category,[137] although rates vary between 2.7%[138] and 15%[139] depending on the population studied. Young patients more frequently develop diffuse lesions which often arise on the background of histologically "normal" gastric mucosa. It is postulated that genetic factors may be more important in EOGC than in older patients as they have less exposure to environmental carcinogens,[140] thus these cancer could provide a key tool in the unraveling the genetic changes in gastric carcinogenesis. *Helicobacter pylori* may still play a role in the development of gastric cancer in young patients, [141, 142] although this is likely to involve a much smaller percentage of patients than in the older age group.

Approximately 10% of young gastric cancer patients have a positive family history,[137] some of which are accounted for by inherited gastric cancer predisposition syndromes, and as discussed under hereditary gastric cancer, the underlying genetic events are not always known but can involve *CDH1* germline mutations.[143, 144] The 90% without a family history emphasizes that the occurrence of gastric cancer in young patients remains largely unexplained.

The clinicopathological features of gastric carcinoma are said to differ between the young and elderly patients and it has been claimed that young patients have a poorer prognosis.[145] Others

Table 1

Characterisitics of EOGC	Reference
more common in females	138,148
diffuse type cancer more common	138,148
often multifocal	151
no intestinal metaplasia	138,148
lack of MSI	149,152,153
infrequent Loss of heterozygosity	153
Low COX2 expression	31
infrequent loss of TFF1 expression	31
no loss of RUNX3	82
gains at chromosomes 17q, 19q and 20q	157

report that tumor staging and prognosis for young patients is similar to older patients and depends on whether the patients undergo a curative resection.[137, 139, 146] Young patients with gastric cancer in the United States are more likely to be black, Asian or Hispanic.[147] Relative to older patients, young patients have a female preponderance, a more frequent occurrence of diffuse cancer and less intestinal metaplasia.[137, 147, 148] This predominance of females is considered by some to be due to hormonal factors.[149] Cancers in young patients are more often multifocal than in older patients [150] as is also seen in HDGC.[133]

Thus early onset gastric cancers are known to have a different clinicopathological profile than conventional gastric carcinomas. This suggests that they represent a separate entity within gastric carcinogenesis and indeed evidence at a molecular genetic level supports this (Table 1). It is known that microsatellite instability which usually occurs at a frequency of 15% in older gastric carcinomas is consistently absent in young patients [148, 151, 152] and this is despite analysis of distal tumors (where MSI is usually commoner) and inclusion of mixed and intestinal type tumors (diffuse tumors generally have less MSI).[153] However, it may be possible that geographical factors play a role.[154] A lack of microsatellite instability excludes the mutator phenotype as an important predisposing factor in the development of early-onset gastric cancer. This contrasts with the situation in colorectal cancer where 58% of patients without HNPCC aged under 35 years showed evidence of microsatellite instability.[155] EOGC also contrasts with colorectal cancer with respect to the tumor suppressor gene *APC* which causes the familial adenomatosis polyposis syndrome. The role of *APC* in EOGC is limited and nuclear expression of β-catenin has not been found to differ between EOGC and conventional gastric cancers.[30]

The molecular expression profile of EOGC and conventional gastric cancers have been found to differ and EOGC have a COX-2 Low, TFF-1 expressing phenotype.[30] A higher incidence of aberrant E-Cadherin expression in EOGC regardless of histological type [148] has also been reported, although a more recent report which compared EOGC with conventional cancers showed that aberrant expression of E-Cadherin correlated significantly with diffuse type.[30] The expression of low molecular weight isoforms of cyclin E are also known to differ between EOGC and conventional cancers, being present in 35% of EOGCs, compared to in 8% of conventional gastric cancers and 4% of stump cancers. In addition, immunohistochemical staining of low molecular weight isoforms of cyclin E were found to be an independent positive prognostic indicator in early-onset gastric cancer (unpublished data).

Recent literature regarding *RUNX3* has excluded it as having a tumor suppressor function in EOGC,[81] although as some of the cell lines used in this study were from conventional gastric cancers, the implications may be more far-reaching and include conventional gastric cancer. Gains at chromosomes 17q, 19q and 20q have been found in EOGC with comparative genomic hybridization [156] and LOH findings have also shown that losses are infrequent in EOGC. [152]

As we can see, EOGCs differ from conventional gastric cancers, not only at a clinicopathological level, but also at a molecular genetic level. If this is indeed due to the fact that the environment plays a smaller role in the triggering of the carcinogenic pathway, the investigation of this group of cancers may reveal genetic changes which assist in the task of putting forward a multistep pathway for gastric cancer.

Future Prospectives

In summary, observations of human cancers and animal models implicate numerous genetic changes in gastric cancer. However, the multistep pathway of carcinogenesis which occurs in some epithelial cancers and which has allowed accurate clinical and pathologic characterization is not yet elucidated in gastric cancer. Gastric cancer exhibits heterogeneity in histopathology and molecular changes that have impeded the uncovering of a temporal molecular pathway. Gastric cancers often occur without any consistent mutational abnormality and with a considerable variation in pathogenesis ranging from a stepwise progression of changes (gastritis -> metaplasia -> dysplasia -> invasive carcinoma) to tumors arising in the absence of a precursor lesion.

Further study of hereditary gastric cancers and early onset gastric cancer as unique subsets of gastric cancer may aid us in the search for a gastric cancer pathway. The rarity of hereditary gastric cancer often hampers research in this field. On the other hand, early-onset gastric cancers, although relatively scarce, provide an ample number of cancers if they can be collected at a nationwide level. Recent developments of techniques adapted to paraffin material will maximize the number of cancers available for research and the use of SNP Chips, expression arrays, kinase arrays and other new technologies, combined with EOGC material may set us well on the road to unraveling gastric carcinogenesis.

Abbreviations

World Health Organisation (WHO), early-onset gastric cancer (EOGC), fibroblast growth factor receptors (FGFR), epidermal growth factor (EGF), hepatocyte growth factor (HGF), Adenomatous polyposis coli (APC), transforming growth factor (TGF), bone morphogenetic protein (BMP), Trefoil factor 1 (TFF1), signal transducers and activators of transcription (STAT), Src-homology tyrosine phosphatase 2 (SHP2), suppressor of cytokine signalling-1 (SOCS-1), Protection of Telomeres-1 (POT1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), melanoma inhibitory activity (MIA), matrix metalloproteinase-10 (MMP-10), microsatellite instability (MSI), hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), hereditary diffuse gastric cancer (HDGC),

References

1. Parkin, D.M., et al., *Estimating the world cancer burden: Globocan 2000.* Int J Cancer, 2001. **94**(2): p. 153-6.
2. 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**: p. 31-49.
3. Hamilton, S.R., L.A. Aaltonen, and (Eds.), *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Digestive System.* IARC Press: Lyon 2000.
4. Schlemper, R.J., et al., *The Vienna classification of gastrointestinal epithelial neoplasia.* Gut, 2000. **47**(2): p. 251-5.
5. Forman, D., et al., *Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation.* Bmj, 1991. **302**(6788): p. 1302-5.
6. Parsonnet, J., et al., *Helicobacter pylori infection and the risk of gastric carcinoma.* N Engl J Med, 1991. **325**(16): p. 1127-31.
7. Suerbaum, S. and P. Michetti, *Helicobacter pylori infection.* N Engl J Med, 2002. **347**(15): p. 1175-86.
8. Craanen, M.E., et al., *Intestinal metaplasia and Helicobacter pylori: an endoscopic bioptic study of the gastric antrum.* Gut, 1992. **33**(1): p. 16-20.
9. Watanabe, T., et al., *Helicobacter pylori infection induces gastric cancer in mongolian gerbils.* Gastroenterology, 1998. **115**(3): p. 642-8.
10. El-Omar, E.M., et al., *Interleukin-1 polymorphisms associated with increased risk of gastric cancer.* Nature, 2000. **404**(6776): p. 398-402.
11. McColl, K.E., *When saliva meets acid: chemical warfare at the oesophagogastric junction.* Gut, 2005. **54**(1): p. 1-3.
12. Figueiredo, C., et al., *Helicobacter pylori and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma.* J Natl Cancer Inst, 2002. **94**(22): p. 1680-7.
13. Machado, J.C., et al., *A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma.* Gastroenterology, 2003. **125**(2): p. 364-71.
14. Stoicov, C., et al., *Molecular biology of gastric cancer: Helicobacter infection and gastric adenocarcinoma: bacterial and host factors responsible for altered growth signaling.* Gene, 2004. **341**: p. 1-17.
15. van Grieken, N.C., et al., *Helicobacter pylori-related and -non-related gastric cancers do not differ with respect to chromosomal aberrations.* J Pathol, 2000. **192**(3): p. 301-6.
16. Chen, S.Y., et al., *Modification effects of GSTM1, GSTT1 and CYP2E1 polymorphisms on associations between raw salted food and incomplete intestinal metaplasia in a high-risk area of stomach cancer.* Int J Cancer, 2004. **108**(4): p. 606-12.
17. Rugge, M. and R.M. Genta, *Epstein-Barr virus: a possible accomplice in gastric oncogenesis.* J Clin Gastroenterol, 1999. **29**(1): p. 3-5.
18. Offerhaus, G.J., et al., *Mortality caused by stomach cancer after remote partial gastrectomy for benign conditions: 40 years of follow up of an Amsterdam cohort of 2633 postgastrectomy patients.* Gut, 1988. **29**(11): p. 1588-90.
19. Ushijima, T. and M. Sasako, *Focus on gastric cancer.* Cancer Cell, 2004. **5**(2): p. 121-5.

20. Dicken, B.J., et al., *Gastric adenocarcinoma: review and considerations for future directions.* Ann Surg, 2005. **241**(1): p. 27-39.
21. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
22. Hara, T., et al., *Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization.* Lab Invest, 1998. **78**(9): p. 1143-53.
23. Hattori, Y., et al., *K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes.* Proc Natl Acad Sci U S A, 1990. **87**(15): p. 5983-7.
24. Park, J.B., et al., *Amplification, overexpression, and rearrangement of the erbB-2 protooncogene in primary human stomach carcinomas.* Cancer Res, 1989. **49**(23): p. 6605-9.
25. Yokota, J., 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. **2**(3): p. 283-7.
26. Lee, J.H., et al., *A novel germ line juxtamembrane Met mutation in human gastric cancer.* Oncogene, 2000. **19**(43): p. 4947-53.
27. Brembeck, F.H., et al., *The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice.* Cancer Res, 2003. **63**(9): p. 2005-9.
28. Park, W.S., et al., *Frequent somatic mutations of the beta-catenin gene in intestinal-type gastric cancer.* Cancer Res, 1999. **59**(17): p. 4257-60.
29. Woo, D.K., et al., *Altered expression and mutation of beta-catenin gene in gastric carcinomas and cell lines.* Int J Cancer, 2001. **95**(2): p. 108-13.
30. Milne, A.N., et al., *Early Onset Gastric Cancers have a different molecular expression profile than conventional gastric cancers.* Modern Pathol, (In Press).
31. Kozma, L., et al., *C-myc amplification and cluster analysis in human gastric carcinoma.* Anticancer Res, 2001. **21**(1B): p. 707-10.
32. Wang, T.C., et al., *Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer.* Gastroenterology, 2000. **118**(1): p. 36-47.
33. Zavros, Y., et al., *Chronic gastritis in the hypochlorhydric gastrin-deficient mouse progresses to adenocarcinoma.* Oncogene, 2005. **24**(14): p. 2354-66.
34. Mimuro, H., et al., *Grb2 is a key mediator of helicobacter pylori CagA protein activities.* Mol Cell, 2002. **10**(4): p. 745-55.
35. Tsutsumi, R., et al., *Attenuation of Helicobacter pylori CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase.* J Biol Chem, 2003. **278**(6): p. 3664-70.
36. van Rees, B.P., et al., *Cyclooxygenase-2 expression during carcinogenesis in the human stomach.* J Pathol, 2002. **196**(2): p. 171-9.
37. Fu, S., et al., *Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in Helicobacter pylori gastritis.* Gastroenterology, 1999. **116**(6): p. 1319-29.
38. Sung, J.J., et al., *Cyclooxygenase-2 expression in Helicobacter pylori-associated premalignant and malignant gastric lesions.* Am J Pathol, 2000. **157**(3): p. 729-35.
39. Saukkonen, K., et al., *Cyclooxygenase-2 and gastric carcinogenesis.* Apmis, 2003. **111**(10): p. 915-25.
40. Caivano, M., et al., *The induction of cyclooxygenase-2 mRNA in macrophages is biphasic and requires both CCAAT enhancer-binding protein beta (C/EBP beta) and C/EBP delta transcription factors.* J Biol Chem, 2001. **276**(52): p. 48693-701.
41. Sankpal, N.V., et al., *Overexpression of CEBPbeta correlates with decreased TFF1 in gastric cancer.* Oncogene, 2006. **25**(4): p. 643-9.

42. Reis, C.A., et al., *Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression*. Cancer Res, 1999. **59**(5): p. 1003-7.
43. Mutoh, H., et al., *Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice*. Biochem Biophys Res Commun, 2002. **294**(2): p. 470-9.
44. Silberg, D.G., et al., *Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice*. Gastroenterology, 2002. **122**(3): p. 689-96.
45. Almeida, R., et al., *Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas*. J Pathol, 2003. **199**(1): p. 36-40.
46. Sato, F. and S.J. Meltzer, *CpG island hypermethylation in progression of esophageal and gastric cancer*. Cancer, 2005.
47. Kaneda, A., et al., *Identification of silencing of nine genes in human gastric cancers*. Cancer Res, 2002. **62**(22): p. 6645-50.
48. Waki, T., et al., *Promoter methylation status of E-cadherin, hMLH1, and p16 genes in nonneoplastic gastric epithelia*. Am J Pathol, 2002. **161**(2): p. 399-403.
49. Hmadcha, A., et al., *Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production*. J Exp Med, 1999. **190**(11): p. 1595-604.
50. Sato, K., et al., *Analysis of genetic and epigenetic alterations of the PTEN gene in gastric cancer*. Virchows Arch, 2002. **440**(2): p. 160-5.
51. Brito, M.J., et al., *Expression of p53 in early (T1) gastric carcinoma and precancerous adjacent mucosa*. Gut, 1994. **35**(12): p. 1697-700.
52. Shim, Y.H., G.H. Kang, and J.Y. Ro, *Correlation of p16 hypermethylation with p16 protein loss in sporadic gastric carcinomas*. Lab Invest, 2000. **80**(5): p. 689-95.
53. Kang, G.H., et al., *Epstein-barr virus-positive gastric carcinoma demonstrates frequent aberrant methylation of multiple genes and constitutes CpG island methylator phenotype-positive gastric carcinoma*. Am J Pathol, 2002. **160**(3): p. 787-94.
54. Nakatsuru, S., et al., *Somatic mutations of the APC gene in precancerous lesion of the stomach*. Hum Mol Genet, 1993. **2**(9): p. 1463-5.
55. Nakatsuru, S., et al., *Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma*. Hum Mol Genet, 1992. **1**(8): p. 559-63.
56. Ogasawara, S., et al., *Lack of mutations of the adenomatous polyposis coli gene in oesophageal and gastric carcinomas*. Virchows Arch, 1994. **424**(6): p. 607-11.
57. Kusano, M., et al., *Absence of microsatellite instability and germline mutations of E-cadherin, APC and p53 genes in Japanese familial gastric cancer*. Tumour Biol, 2001. **22**(4): p. 262-8.
58. Lee, J.H., et al., *Inverse relationship between APC gene mutation in gastric adenomas and development of adenocarcinoma*. Am J Pathol, 2002. **161**(2): p. 611-8.
59. Smits, R., et al., *E-cadherin and adenomatous polyposis coli mutations are synergistic in intestinal tumor initiation in mice*. Gastroenterology, 2000. **119**(4): p. 1045-53.
60. Park, K., et al., *Genetic changes in the transforming growth factor beta (TGF-beta) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-beta*. Proc Natl Acad Sci U S A, 1994. **91**(19): p. 8772-6.
61. Uchino, S., et al., *Frequent loss of heterozygosity at the DCC locus in gastric cancer*. Cancer Res, 1992. **52**(11): p. 3099-102.
62. Xu, X., et al., *Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice*. Oncogene, 2000. **19**(15): p. 1868-74.

63. Wen, X.Z., et al., *BMP-2 modulates the proliferation and differentiation of normal and cancerous gastric cells.* Biochem Biophys Res Commun, 2004. **316**(1): p. 100-6.
64. Wen, X.Z., et al., *Frequent epigenetic silencing of the bone morphogenetic protein 2 gene through methylation in gastric carcinomas.* Oncogene, 2005.
65. Lefebvre, O., et al., *Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein.* Science, 1996. **274**(5285): p. 259-62.
66. Carvalho, R., et al., *Loss of heterozygosity and promoter methylation, but not mutation, may underlie loss of TFF1 in gastric carcinoma.* Lab Invest, 2002. **82**(10): p. 1319-26.
67. Saukkonen, K., et al., *Cyclooxygenase-2 expression and effect of celecoxib in gastric adenomas of trefoil factor 1-deficient mice.* Cancer Res, 2003. **63**(12): p. 3032-6.
68. Tebbutt, N.C., et al., *Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice.* Nat Med, 2002. **8**(10): p. 1089-97.
69. Judd, L.M., et al., *Gastric cancer development in mice lacking the SHP2 binding site on the IL-6 family co-receptor gp130.* Gastroenterology, 2004. **126**(1): p. 196-207.
70. Jenkins, B.J., et al., *Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling.* Nat Med, 2005. **11**(8): p. 845-52.
71. To, K.F., et al., *Constitutional activation of IL-6-mediated JAK/STAT pathway through hypermethylation of SOCS-1 in human gastric cancer cell line.* Br J Cancer, 2004. **91**(7): p. 1335-41.
72. Oshimo, Y., et al., *Epigenetic inactivation of SOCS-1 by CpG island hypermethylation in human gastric carcinoma.* Int J Cancer, 2004. **112**(6): p. 1003-9.
73. Ohta, M., et al., *The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers.* Cell, 1996. **84**(4): p. 587-97.
74. Baffa, R., et al., *Loss of FHIT expression in gastric carcinoma.* Cancer Res, 1998. **58**(20): p. 4708-14.
75. Fujishita, T., et al., *Development of spontaneous tumours and intestinal lesions in Fhit gene knockout mice.* Br J Cancer, 2004. **91**(8): p. 1571-4.
76. Drusco, A., et al., *Knockout mice reveal a tumor suppressor function for Testin.* Proc Natl Acad Sci U S A, 2005. **102**(31): p. 10947-51.
77. Zanesi, N., et al., *The tumor spectrum in FHIT-deficient mice.* Proc Natl Acad Sci U S A, 2001. **98**(18): p. 10250-5.
78. Li, Q.L., et al., *Causal relationship between the loss of RUNX3 expression and gastric cancer.* Cell, 2002. **109**(1): p. 113-24.
79. Levanon, D., et al., *The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons.* Embo J, 2002. **21**(13): p. 3454-63.
80. Levanon, D., et al., *Runx3 knockouts and stomach cancer.* EMBO Rep, 2003. **4**(6): p. 560-4.
81. Carvalho, R., et al., *Exclusion of RUNX3 as a tumour-suppressor gene in early-onset gastric carcinomas.* Oncogene, 2005. **24**(56): p. 8252-8.
82. Eguchi, H., et al., *Helicobacter pylori increases proteasome-mediated degradation of p27(kip1) in gastric epithelial cells.* Cancer Res, 2003. **63**(15): p. 4739-46.
83. Eguchi, H., et al., *P27kip1 regulates the apoptotic response of gastric epithelial cells to Helicobacter pylori.* Gut, 2004. **53**(6): p. 797-804.
84. Kuzushita, N., et al., *p27kip1 deficiency confers susceptibility to gastric carcinogenesis in Helicobacter pylori-infected mice.* Gastroenterology, 2005. **129**(5): p. 1544-56.

85. Koshida, Y., M. Saegusa, and I. Okayasu, *Apoptosis, cell proliferation and expression of Bcl-2 and Bax in gastric carcinomas: immunohistochemical and clinicopathological study*. Br J Cancer, 1997. **75**(3): p. 367-73.
86. Cai, X., et al., *Overcoming Fas-mediated apoptosis accelerates Helicobacter-induced gastric cancer in mice*. Cancer Res, 2005. **65**(23): p. 10912-20.
87. Mannick, J.B., et al., *Fas-induced caspase denitrosylation*. Science, 1999. **284**(5414): p. 651-4.
88. Galmiche, A., et al., *The N-terminal 34 kDa fragment of Helicobacter pylori vacuolating cytotoxin targets mitochondria and induces cytochrome c release*. Embo J, 2000. **19**(23): p. 6361-70.
89. Kondo, T., et al., *Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma*. Cancer Res, 2004. **64**(2): p. 523-9.
90. Tuccillo, C., et al., *Vascular endothelial growth factor and neo-angiogenesis in H. pylori gastritis in humans*. J Pathol, 2005. **207**(3): p. 277-84.
91. Kitadai, Y., et al., *Transfection of interleukin-8 increases angiogenesis and tumorigenesis of human gastric carcinoma cells in nude mice*. Br J Cancer, 1999. **81**(4): p. 647-53.
92. Monig, S.P., et al., *Expression of MMP-2 is associated with progression and lymph node metastasis of gastric carcinoma*. Histopathology, 2001. **39**(6): p. 597-602.
93. Aung, P.P., et al., *Systematic search for gastric cancer-specific genes based on SAGE data: melanoma inhibitory activity and matrix metalloproteinase-10 are novel prognostic factors in patients with gastric cancer.* Oncogene, 2005.
94. Sano, T., et al., *Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17p in human gastric carcinomas*. Cancer Res, 1991. **51**(11): p. 2926-31.
95. Carvalho, B., et al., *Substantial reduction of the gastric carcinoma critical region at 6q16.3-q23.1*. Genes Chromosomes Cancer, 1999. **26**(1): p. 29-34.
96. El-Rifai, W., et al., *Consistent genetic alterations in xenografts of proximal stomach and gastro-esophageal junction adenocarcinomas*. Cancer Res, 1998. **58**(1): p. 34-7.
97. Yustein, A.S., et al., *Allelotype of gastric adenocarcinoma*. Cancer Res, 1999. **59**(7): p. 1437-41.
98. Wu, C.W., et al., *Clinical implications of chromosomal abnormalities in gastric adenocarcinomas*. Genes Chromosomes Cancer, 2002. **35**(3): p. 219-31.
99. Baik, S.C., et al., *Increased oxidative DNA damage in Helicobacter pylori-infected human gastric mucosa*. Cancer Res, 1996. **56**(6): p. 1279-82.
100. Sobala, G.M., et al., *Effect of eradication of Helicobacter pylori on gastric juice ascorbic acid concentrations*. Gut, 1993. **34**(8): p. 1038-41.
101. Touati, E., et al., *Chronic Helicobacter pylori infections induce gastric mutations in mice*. Gastroenterology, 2003. **124**(5): p. 1408-19.
102. Peltomaki, P., et al., *Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome*. Cancer Res, 1993. **53**(24): p. 5853-5.
103. Hayden, J.D., et al., *The role of microsatellite instability in gastric carcinoma*. Gut, 1998. **42**(2): p. 300-3.
104. Wu, M.S., et al., *Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes*. Genes Chromosomes Cancer, 2000. **27**(4): p. 403-11.
105. Wu, M.S., et al., *Infrequent hMSH2 mutations in sporadic gastric adenocarcinoma with microsatellite instability*. Cancer Lett, 1997. **112**(2): p. 161-6.
106. Fleisher, A.S., et al., *Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability*. Cancer Res, 1999. **59**(5): p. 1090-5.

107. Leung, S.Y., et al., *hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability.* Cancer Res, 1999. **59**(1): p. 159-64.
108. Machado, J.C., et al., *E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas.* Lab Invest, 1999. **79**(4): p. 459-65.
109. Nardone, G., A. Rocco, and P. Malfertheiner, *Review article: helicobacter pylori and molecular events in precancerous gastric lesions.* Aliment Pharmacol Ther, 2004. **20**(3): p. 261-70.
110. Eslick, G.D., et al., *Association of Helicobacter pylori infection with gastric carcinoma: a meta-analysis.* Am J Gastroenterol, 1999. **94**(9): p. 2373-9.
111. Terres, A.M., et al., *H pylori infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control.* J Clin Pathol, 1998. **51**(5): p. 410-2.
112. Aggarwal, A., et al., *Topological and functional discovery in a gene coexpression meta-network of gastric cancer.* Cancer Res, 2006. **66**(1): p. 232-41.
113. Sokoloff, B., *Predisposition to cancer in the Bonaparte family.* Am J Surg, 1938. **40**: p. 637-8.
114. Olivier, M., et al., *Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype.* Cancer Res, 2003. **63**(20): p. 6643-50.
115. *Cancer risks in BRCA2 mutation carriers.The Breast Cancer Linkage Consortium.* J Natl Cancer Inst, 1999. **91**(15): p. 1310-6.
116. Jakubowska, A., et al., *BRCA2 gene mutations in families with aggregations of breast and stomach cancers.* Br J Cancer, 2002. **87**(8): p. 888-91.
117. Lynch, H.T., T. Smyrk, and J.F. Lynch, *Overview of natural history, pathology, molecular genetics and management of HNPCC (Lynch Syndrome).* Int J Cancer, 1996. **69**(1): p. 38-43.
118. Aaltonen, L.A., et al., *Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients.* Cancer Res, 1994. **54**(7): p. 1645-8.
119. Offerhaus, G.J., M.M. Entius, and F.M. Giardiello, *Upper gastrointestinal polyps in familial adenomatous polyposis.* Hepatogastroenterology, 1999. **46**(26): p. 667-9.
120. Giardiello, F.M., et al., *Increased risk of cancer in the Peutz-Jeghers syndrome.* N Engl J Med, 1987. **316**(24): p. 1511-4.
121. Lim, W., et al., *Relative frequency and morphology of cancers in STK11 mutation carriers.* Gastroenterology, 2004. **126**(7): p. 1788-94.
122. Oliveira, C., R. Seruca, and F. Carneiro, *Genetics, pathology and clinics of familial gastric cancer.* Int J Surg Pathol, 2006. **In Press**.
123. Oliveira, C., et al., *E-Cadherin (CDH1) and p53 rather than SMAD4 and Caspase-10 germline mutations contribute to genetic predisposition in Portuguese gastric cancer patients.* Eur J Cancer, 2004. **40**(12): p. 1897-903.
124. Grunwald, G.B., *The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules.* Curr Opin Cell Biol, 1993. **5**(5): p. 797-805.
125. Guilford, P., et al., *E-cadherin germline mutations in familial gastric cancer.* Nature, 1998. **392**(6674): p. 402-5.
126. Pharoah, P.D., P. Guilford, and C. Caldas, *Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families.* Gastroenterology, 2001. **121**(6): p. 1348-53.
127. Caldas, C., et al., *Familial gastric cancer: overview and guidelines for management.* J Med Genet, 1999. **36**(12): p. 873-80.

128. Berx, G., et al., *Mutations of the human E-cadherin (CDH1) gene.* Hum Mutat, 1998. **12**(4): p. 226-37.
129. Grady, W.M., et al., *Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer.* Nat Genet, 2000. **26**(1): p. 16-7.
130. McColl, K.E. and E. El-Omar, *E-cadherin germline mutations and risk of gastric cancer.* Gastroenterology, 2002. **123**(4): p. 1406; author reply 1406-7.
131. Fitzgerald, R.C. and C. Caldas, *Clinical implications of E-cadherin associated hereditary diffuse gastric cancer.* Gut, 2004. **53**(6): p. 775-8.
132. Charlton, A., et al., *Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone.* Gut, 2004. **53**(6): p. 814-20.
133. Carneiro, F., et al., *Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening.* J Pathol, 2004. **203**(2): p. 681-7.
134. Shaw, D., et al., *Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy?* Gut, 2005. **54**(4): p. 461-8.
135. Huntsman, D.G., et al., *Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations.* N Engl J Med, 2001. **344**(25): p. 1904-9.
136. Chun, Y.S., et al., *Germline E-cadherin gene mutations: is prophylactic total gastrectomy indicated?* Cancer, 2001. **92**(1): p. 181-7.
137. Kokkola, A. and P. Sipponen, *Gastric carcinoma in young adults.* Hepatogastroenterology, 2001. **48**(42): p. 1552-5.
138. Umeyama, K., et al., *Gastric carcinoma in young adults in Japan.* Anticancer Res, 1982. **2**(5): p. 283-6.
139. Ramos-De la Medina, A., et al., *Clinicopathologic characteristics of gastric cancer in a young patient population.* J Gastrointest Surg, 2004. **8**(3): p. 240-4.
140. Correa, P. and Y.H. Shiao, *Phenotypic and genotypic events in gastric carcinogenesis.* Cancer Res, 1994. **54**(7 Suppl): p. 1941s-1943s.
141. Rugge, M., et al., *Patients younger than 40 years with gastric carcinoma: Helicobacter pylori genotype and associated gastritis phenotype.* Cancer, 1999. **85**(12): p. 2506-11.
142. Koshida, Y., et al., *Association of Helicobacter pylori-dependent gastritis with gastric carcinomas in young Japanese patients: histopathological comparison of diffuse and intestinal type cancer cases.* Histopathology, 2000. **37**(2): p. 124-30.
143. Suriano, G., et al., *Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands.* Hum Mol Genet, 2003. **12**(5): p. 575-82.
144. Suriano, G., et al., *Characterization of a recurrent germ line mutation of the E-cadherin gene: implications for genetic testing and clinical management.* Clin Cancer Res, 2005. **11**(15): p. 5401-9.
145. Theuer, C.P., et al., *Gastric adenocarcinoma in patients 40 years of age or younger.* Am J Surg, 1996. **172**(5): p. 473-6; discussion 476-7.
146. Medina-Franco, H., M.J. Heslin, and R. Cortes-Gonzalez, *Clinicopathological characteristics of gastric carcinoma in young and elderly patients: a comparative study.* Ann Surg Oncol, 2000. **7**(7): p. 515-9.
147. Matley, P.J., et al., *Gastric carcinoma in young adults.* Ann Surg, 1988. **208**(5): p. 593-6.
148. Lim, S., et al., *Alteration of E-cadherin-mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers.* Histopathology, 2003. **42**(2): p. 128-136.

149. Maeta, M., et al., *Gastric cancer in the young, with special reference to 14 pregnancy-associated cases: analysis based on 2,325 consecutive cases of gastric cancer.* J Surg Oncol, 1995. **58**(3): p. 191-195.
150. Furukawa, H., et al., *Multifocal gastric cancer in patients younger than 50 years of age.* Eur Surg Res, 1989. **21**(6): p. 313-8.
151. Hayden, J.D., et al., *Assessment of microsatellite alterations in young patients with gastric adenocarcinoma.* Cancer, 1997. **79**(4): p. 684-687.
152. Carvalho, R., et al., *Early-onset gastric carcinomas display molecular characteristics distinct from gastric carcinomas occurring at a later age.* J Pathol, 2004. **204**(1): p. 75-83.
153. Seruca, R. and M. Sobrinho-Simoes, *Assessment of microsatellite alterations in young patients with gastric adenocarcinoma.* Cancer, 1997. **80**(7): p. 1358-1360.
154. Hayden, J.D., et al., *A comparison of microsatellite instability in early onset gastric carcinomas from relatively low and high incidence European populations.* Int J Cancer, 2000. **85**(2): p. 189-91.
155. Liu, B., et al., *Genetic instability occurs in the majority of young patients with colorectal cancer.* Nat Med, 1995. **1**(4): p. 348-52.
156. Varis, A., et al., *DNA copy number changes in young gastric cancer patients with special reference to chromosome 19.* Br.J.Cancer, 2003. **88**(12): p. 1914-1919.