Molecular alterations in gastro-esophageal carcinogenesis

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CHAPTER 1

General Introduction
GENERAL INTRODUCTION

1. ADENOCARCINOMA OF THE STOMACH
Gastric cancer is the result of a long multistage carcinogenic process where environmental and genetic factors interact (1). Despite its declining incidence in the Western world, it remains one of the leading causes of cancer-related death worldwide (2,3). The prognosis of gastric carcinoma is poor since most patients become symptomatic when the disease is already in an advanced stage. Patients with so-called early gastric cancer, limited to the mucosa or submucosa, have an excellent prognosis and can be detected by surveillance of high-risk groups (4).

Histology
Histologically, more than 90% of gastric cancers are adenocarcinomas. Over time, many histological classification systems have been developed. The Laurén classification has proven useful in evaluating the natural history of gastric carcinoma, especially with regard to its association with environmental factors (5). According to this classification, tumors are divided in two distinctive types: 'intestinal' or 'diffuse'. Intestinal carcinomas are characterized by recognizable glands and cohesion between tumor cells. They typically arise in a background of chronic atrophic gastritis and intestinal metaplasia. Diffuse carcinomas consist of poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation. The cells usually appear round and small, often with signet cell morphology. Diffuse tumors typically arise in histologically 'normal-appearing' gastric mucosa. Tumors that contain approximately equal quantities of intestinal and diffuse components are called 'mixed carcinomas'. If a carcinoma is too undifferentiated to fit neatly into either category, it is classified as 'indeterminate'.

Precursor lesions
Studies of specimens obtained at surgery or autopsy have suggested that gastric cancers often develop in the context of other conditions. Chronic atrophic gastritis and its associated abnormality, intestinal metaplasia, are the lesions most closely linked to an increased risk of gastric cancer, specifically the intestinal type (6). Atrophic gastritis usually begins as a multifocal process in the distal stomach (7). As foci coalesce, a state of reduced acid production results, which may progress to metaplasia, dysplasia, and ultimately carcinoma (8). It is postulated that these precursor lesions of the intestinal type of stomach cancer represent the morphological substrates of a multistep tumor progression model, comparable to the adenoma-carcinoma sequence of the colorectum (9).

Epidemiology
With the exception of carcinomas of the gastric cardia (see below), the incidence of gastric adenocarcinoma in the Western world has been declining over the past several decades (1). The male to female ratio is approximately 3:2 for the intestinal type whereas the diffuse type shows no obvious difference in prevalence between males and females.
Significant geographic variability in incidence is observed. The incidence is highest in Japan, China, South America and Eastern Europe (10). There is about a 20-fold difference in the incidence rates when comparing the rates in Japan with those of some white populations in the Western world. In high-risk areas a predominance of the intestinal type adenocarcinoma occurs, while the diffuse type is relatively more common in low-risk areas (11). Gastric carcinoma is extremely rare below the age of 30; thereafter its incidence increases steadily. The highest incidence is found in the oldest age groups. In older patients the intestinal type is more predominant. In younger patients, particularly those below the age of 45, the diffuse type is more predominant (12). In this age group the disease frequently has hereditary characteristics.

Pathogenesis and risk factors
The pathogenesis of gastric cancer is complex and incompletely understood, but diet, infections and genetic factors are involved (13). Epidemiological studies in different populations show that the most consistent association is diet. This is especially true for the intestinal type carcinomas. There is strong evidence that high intake of fresh fruits and vegetables reduces the risk of developing gastric cancer, presumably due to their antioxidant effects. High intake of salt and foods rich in n-nitroso compounds are likely to increase the risk of gastric cancer and its precursor lesions (14). Intake of smoked or cured meats or fish and pickled vegetables are also associated with a higher risk of gastric cancer. A large part of the decline in incidence of intestinal type gastric cancer in the Western world in the last decades can be attributed to the introduction of the refrigerator and its associated change in preservation of foods. Overall, the role of alcohol and tobacco smoking and other environmental factors has not been well established yet and the potential mechanisms of action are not well understood.

Helicobacter pylori
Epidemiological studies have consistently demonstrated an association between H. pylori and the risk of gastric carcinoma (15). Prospective serologic studies have reported that persons with H. pylori have an approximately three- to sixfold higher risk of gastric cancer than those without infection (16-18). In 1994, H. pylori was classified as a group-I carcinogen by the International Agency for Research on cancer (19). In a recent prospective follow-up study of 1526 Japanese patients with duodenal ulcers, gastric ulcers, gastric hyperplasia and non-ulcer dyspepsia, gastric cancer only developed in patients (2.9 percent) with H. pylori infection whereas none of the patients without infection developed gastric carcinoma (20).

H. pylori has been recognized as the most important factor in the pathogenesis of peptic ulcer disease. Interestingly, patients with peptic ulcers do not have an increased risk for the subsequent development of gastric cancer (21). Therefore, the development of gastric cancer in patients with H. pylori gastritis seems to be largely dependent on host (genetic) factors. Recently, it has been shown that certain polymorphisms in the interleukin-1 (IL-1) genes that might modify the host response to inflammatory stimuli, are associated with an increased risk of gastric cancer (22,23). The precise role of H. pylori in gastric carcinogenesis remains unclear.
although it is associated with the development of chronic atrophic gastritis. Analysis of molecular alterations in gastric carcinomas has not revealed any specific alterations associated with *H. pylori* infection (24,25). Therefore, the role of *H. pylori* in the process of gastric carcinogenesis seems limited to the induction of chronic inflammation and increased cell proliferation.

*Epstein-Barr virus*

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus with well-established associations with endemic Burkitt's lymphoma (26), nasopharyngeal carcinoma (27) and opportunistic B-cell lymphomas in immunodeficiency (28). A subset of gastric carcinomas, known as lymphoepithelioma-like gastric carcinoma (LELC) are known to harbor the EBV genome in a high proportion of cases (29-31). Furthermore, conventional gastric adenocarcinomas were shown to contain EBV in a small proportion of cases (approximately 8-10%) (29-32). In contrast to Burkitt's lymphoma and nasopharyngeal carcinoma, the causal role of EBV in gastric carcinogenesis is not well understood. The latency type of EBV infection in gastric carcinoma is different from the known latency types as seen in these malignancies. The latent membrane protein-1 (LMP-1), one of the known transforming oncoproteins of EBV is usually not expressed in EBV-carrying gastric carcinomas. Nevertheless, findings such as the clonal nature of EBV in GC cells and the presence of EBV in nearly all of the cancer cells in EBV positive GC are supportive of a causal role of EBV in gastric carcinogenesis (29,30,32). Furthermore, the transforming *BARF1* gene was found to be expressed frequently in EBV-positive gastric carcinomas (33). In addition, it was recently shown that the expression of *RUNX3*, a gene causally related to stomach cancer is induced by the EBV transcription factor EBNA-2 (34,35).

*Gastric stump carcinoma*

Remote partial gastrectomy is a premalignant condition (36,37). After peptic ulcer surgery, patients enter an accelerated neoplastic process, similar to that proposed for the intestinal type of gastric cancer in the non-operated stomach. There is a steady increase in gastric cancer risk with increasing length of the postoperative interval (38). The mechanism for the increased cancer risk is incompletely understood but bile reflux is thought to play an important role in this process. In the long term after partial gastrectomy, atrophy with intestinal metaplasia and dysplasia becomes more frequent and these changes resemble the precursors of the intestinal type of stomach cancer (39,40). Also the cell proliferation kinetics of the premalignant mucosa in the gastric remnant are similar to those observed in the non-operated stomach (41). Therefore it was postulated that gastric stump cancer could provide a suitable model to study the molecular genetics of gastric carcinogenesis in general (42).

*Genetic susceptibility*

Most gastric carcinomas occur sporadically; only 8-10% have an inherited familial component (43). Familial clustering occurs in 12 to 25% with a dominant inheritance pattern (44,45). A small but consistent increased risk has been observed in first-degree relatives of gastric carcinoma patients (46).
Blood group A phenotype is associated with gastric cancer (47,48). *H. pylori* adheres to the Lewis^b^ blood group antigen which may be an important host factor facilitating chronic infection and subsequent cancer risk (49). The above-mentioned IL-1 polymorphisms may be involved in the modulation of the host response as well. Gastric cancers can also develop as part of the hereditary non-polyposis colon cancer syndrome (HNPCC) (50,51). They are mainly intestinal type cancers, without a clear association with *H. pylori* infection. Most of these tumors exhibited microsatellite instability (MSI) (52).

Gastric carcinomas also occur in patients with gastrointestinal polyposis syndromes such as Familial Adenomatous Polyposis (FAP), Juvenile Polyposis, and Peutz-Jeghers syndrome. In general, gastric carcinoma is rare in these settings. Also the exact contribution of the polyposis and the underlying germline mutations of *APC*, *SMAD4* and *LKB1/STK11* to gastric cancer development is as yet unclear. Occasionally, gastric carcinoma develops in families with germline mutations in *ATM5*, *TP53* (Li Fraumeni syndrome) and *BRCA2* (53-56). Germline mutations in the gene encoding the cell adhesion protein E-cadherin (*CDH1*) have also been found. They lead to an autosomal dominant predisposition to gastric carcinoma, referred to as hereditary diffuse gastric carcinoma (HDGC) (57,58). Predisposing germline *CDH1* mutations, generally resulting in truncated proteins, are spread throughout the gene with no apparent hotspots (57-60). HDGC has an age of onset ranging from 14 years upwards and a penetrance of approximately 70% (58,59). Histologically, HDGC tumors are of the diffuse type according to the Laurén classification.

**Molecular alterations in gastric cancer**

The current concept of cancer is that it is the result of an accumulation of generalized and specific molecular genetic alterations in which activated oncogenes and inactivated tumor suppressor genes -sometimes caused by defective proteins involved in DNA repair- lead to growth deregulation at the cellular level (51,61).

**Oncogenes**

Proto-oncogenes, genes normally involved in cell proliferation, may become constitutively activated, leading to abnormal cellular growth and thereby contributing to tumorigenesis. Activation of these genes can be accomplished by amplification, point mutation, or by fusion to other genes or their regulatory elements as a result of chromosomal translocation. Many of these genes encode growth factors, growth factor receptors or other signaling intermediates involved in cell growth. The *K-RAS* proto-oncogene is frequently activated in gastrointestinal tumors. *K-RAS* encodes a small 21 kD GTP-ase that is involved in signal transduction (62). *K-RAS* is known to be oncogenic when mutated or overexpressed. When mutated in codon 12, 13 or 61 the gene encodes a protein with impaired intrinsic GTP-ase activity. As a consequence the protein remains in the active GTP-bound state, resulting in a constitutive activation of K-RAS in the signal transduction pathway. *K-RAS* is not frequently mutated in adenocarcinomas of the stomach (63,64).

Amplification, overexpression and translocation of the *c-MET* gene encoding a tyrosine kinase receptor for the hepatocyte growth factor have been described in
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gastric carcinoma (65). Amplification of c-ERBB2 (HER2/neu), a transmembrane tyrosine kinase receptor oncogene, occurs in approximately 10% of lesions and overexpression is associated with a poor prognosis (66). Other growth factor and receptor signal systems that may be involved include epidermal growth factor, cyclin-E, TGF-α, interleukin-1a, cripto, amphiregulin, platelet-derived growth factor and K-SAM (67,68).

**Tumor suppressor genes**
The loss of genes involved in the regulation of growth inhibition, differentiation and cell death also contributes to tumorigenesis. These mechanisms include cell cycle control, regulation of transcription and induction of apoptosis. According to Knudson’s hypothesis (69) both copies of a tumor suppressor gene need to be inactivated to abrogate its suppressive effects, whereas an activating mutation of one allele of an oncogene is sufficient for its tumorigenic activity. Tumor suppressor genes can become inactivated by point mutations, deletions or gene silencing (e.g. by methylation).

Analysis of loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) have identified several loci with significant allelic loss, indicating that these regions may harbor tumor suppressor genes important in gastric carcinoma.

Common sites of loss include regions on chromosomal arms 1p, 1q, 3p, 4, 5q, 9p, 17p, 18q and 20q (1,68,70-74).

**FHIT**
In gastric cancer, frequent loss of heterozygosity of several regions of chromosomal arm 3p has been reported, suggesting the presence of tumor suppressor loci (75,76). The 3p14 region harbors the fragile histidine triad (FHIT) gene. Abnormal transcripts, deletions, somatic missense mutations and loss of the FHIT protein have been reported in gastric carcinomas (77). Nevertheless, it has remained uncertain whether FHIT is the sole target of the frequent LOH on chromosomal arm 3p in gastric cancer. Other tumor suppressor genes in that region, that are yet to be identified, might be of more importance in gastric carcinogenesis.

**APC**
The APC tumor suppressor gene, located on chromosome 5q21, is mutated in 80% of sporadic colorectal cancer. APC encodes a 312 kD protein and participates as part of a complex in the Wnt/APC/β-catenin signaling pathway that stimulates cell proliferation (51,78). Under physiological circumstances the APC/β-catenin complex is tightly regulated. However, mutations in APC or the β-catenin gene (CTNNB1) can disturb this equilibrium and large amounts of free β-catenin become available. This can then bind and activate the transcription factor TCF-4 resulting in uncontrolled transcription of TCF-4 target genes (79). The c-MYC oncogene, CCND1 and PPAR-δ have been identified as transcriptional targets of this pathway (80-82). Alterations of APC and CTNNB1 have been described in up to 50% of subsets of gastric adenocarcinomas (83-86).
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**p16/MTS-1/INK4a and p14ARF**

The p16/MTS-1/INK4a tumor suppressor gene, located on chromosomal arm 9p21, is frequently inactivated in a variety of tumors (87). The p16 protein acts as an inhibitor of the cyclin D-dependent protein kinases. Reduced expression of p16 results in higher cyclin D-dependent protein kinase activity and therefore in aberrant phosphorylation of Rb, which consequently accelerates cell growth (88). Although loss of p16 protein has been observed in human gastric carcinoma (GC), genetic alterations of p16 are infrequent in gastric carcinoma (89,90). In contrast, a high frequency of p16 hypermethylation was reported in GC and this seems to be correlated with loss of p16 protein expression, which occurs in up to 51% in some series (91,92).

p14ARF is another tumor suppressor protein which is generated through an alternative splicing process that replaces the first exon, 1α, of p16INK4a with exon 1β, which is located >15 kb upstream of exon 1α. This tumor suppressor gene was recently shown to be frequently inactivated by homozygous deletion or methylation in gastric cancer cell lines and primary gastric carcinomas, particularly of the diffuse type (93).

**E-Cadherin (CDH1)**

The CDH1 gene is located on chromosomal arm 16q. E-Cadherin is a calcium dependent cell-cell adhesion molecule, which is essential for the formation and maintenance of normal architecture and function of epithelial tissues (94). The adhesive function of E-cadherin is dependent on interaction of its cytoplasmic domain with α, β, and γ-catenin. Loss of function of E-cadherin will not only reduce cellular adhesion but may also result in release of unbound β-catenin, which is an important player in the Wnt-signaling pathway (see above).

Reduced or abnormal E-cadherin expression has been observed in sporadic gastric carcinomas, especially of the diffuse type (95-97). Furthermore, mutations of the gene and abnormalities of its transcripts have been identified in gastric cancer (98). CDH1 splice site alterations lead to exon deletion and -skipping. Large deletions and missense point mutations also occur. Somatic mutations of CDH1 were identified in the diffuse component of mixed tumors, whereas they were absent in the intestinal component (99).

**TP53**

The p53 tumor suppressor protein, encoded by the TP53 tumor suppressor gene on chromosome 17p, is a transcription factor, which is activated in response to DNA damage (100). Upon activation, p53 can induce apoptosis and G1 cell-cycle arrest. Moreover, p53 is directly involved in DNA repair itself (101). Therefore, functional inactivation of p53 leads to loss of three important elements in cell-cycle control. TP53 is frequently inactivated in various types of human cancer. Allelic loss of the TP53 locus on chromosomal arm 17p occurs in >60% of gastric carcinomas and mutations are identified in approximately 30-50%, depending on the mutational screening method and sample sizes (102,103). TP53 mutations have been found in advanced gastric carcinomas but were also identified in lesions as early as intestinal metaplasia (104).
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**DCC, SMAD2, SMAD4**

Like in other digestive tract tumors, allelic loss of the long arm of chromosome 18 is frequently observed in gastric carcinoma and is associated with tumor progression and poor prognosis (70-74,105). This suggests that the 18q region harbors a tumor suppressor gene important for gastric carcinogenesis and tumor progression. Candidate tumor suppressor genes on 18q are DCC, SMAD2 and SMAD4.

The DCC (for deleted in colorectal cancer) tumor suppressor gene encodes a large protein with significant sequence similarity to neural cell adhesion molecules and other related cell surface glycoproteins. Alterations of this gene may interfere with normal cell growth and differentiation by disrupting cell-cell or cell-substrate interactions. Genetic mutations of DCC are rare, both in colorectal and in gastric carcinoma but frequent loss of expression of the DCC protein was recently described in gastric carcinoma (106,107).

SMAD2 and SMAD4 (also known as DPC4, for deleted in pancreatic cancer) are other candidate tumor suppressor genes in this chromosomal region. Both genes participate in the TGF-ß-signaling pathway (108). TGF-ß is a pleiotropic growth factor involved in the regulation of growth of epithelial cells. Germline mutations of SMAD4 have been found in patients with juvenile polyposis. Furthermore, SMAD4 is frequently mutated in pancreatic adenocarcinomas. However, mutations of both SMAD2 and SMAD4 are rare in colorectal and gastric cancer (108-111).

Therefore, the target of the frequent loss of chromosome 18q may be yet another, still unidentified tumor suppressor gene.

**Trefoil Factor Family 1 (TFF1)**

Whereas the chromosomal regions 3p, 5q, 9p, 17p and 18q, harboring known tumor suppressor genes, are commonly deleted in a variety of cancers, mice homozygously deleted for Trefoil Factor Family 1 gene (TFF1), specifically show neoplastic aberrations of the stomach. These mice develop high-grade dysplasia of the gastric mucosa at 5 months of age which progresses to invasive carcinomas in 30% (112). Furthermore, TFF1, located on chromosome 21q, was recently found to be mutated in a subset of human gastric carcinomas (113).

**Mismatch repair genes**

Microsatellite instability (MSI) is a form of genetic instability observed in virtually all tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC) and in a subset of various sporadic tumors, including colorectal, gastric and endometrial cancer (114,115). The majority of HNPCC patients have germline mutations in one of several DNA mismatch repair (MMR) genes (116). Genes involved in the mismatch repair process are hMLH1, hMSH2, hMLH3, hMSH3, hMSH6, PMS1, PMS2 and EXO1. During DNA replication, the products of these genes form complexes that ensure the integrity of the DNA. A mutated MMR gene can lead to a defect MMR mechanism and the accumulation of random DNA errors, especially in repeat sequences, leading to the molecular phenotype of MSI. Defective MMR is thought to promote tumorigenesis by accelerating the accumulation of mutations in oncogenes and tumor suppressor genes. MSI has been observed in a subset of gastric carcinomas up to 44% depending on the group of cases studied and the type and
number of markers examined (117-120). Interestingly, mutations of \textit{hMSH2} and \textit{hMLH1} are infrequent in sporadic tumors with MSI, including gastric carcinoma (121,122). Studies of several MSI-High (i.e. MSI in >40% of the tested markers) tumor types including gastric cancer demonstrated a frequent loss of expression of \textit{hMLH1} and \textit{hMSH2}, despite the lack of identifiable germline or somatic mutations (123-125). Interestingly, it was shown that hypermethylation of the \textit{hMLH1} promoter rather than inactivating mutations appear to underlie the loss of \textit{hMLH1} expression (126,127). MSI-High gastric carcinomas appear to be clinicopathologically distinct and tend to have a better outcome (128,129).

Prostaglandins and Cyclooxygenase

Human trials and animal studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of cancer, especially in the gastrointestinal tract. The best-known targets of NSAIDs are the cyclooxygenase (COX) enzymes that catalyze the rate-limiting step in the conversion of arachidonic acid into prostanoids. The COX-2 isoform is overexpressed in a variety of human malignancies, including gastric cancer (130). Gastric and colon cancer tissues release higher levels of prostaglandin E$_2$ (PGE$_2$) when compared to nonneoplastic mucosa (131,132). This phenomenon seems to be specific for PGE$_2$, since levels of other prostanoids were not increased. PGE$_2$ has been shown to be mechanistically linked to carcinogenesis, since it inhibits apoptosis and enhances growth and motility of colon cancer cells (133,134). An extensive introduction regarding the role of the COX-2 - prostanoid pathway in carcinogenesis of the digestive tract is provided in chapter 6.

2. ADENOCARCINOMA OF THE GASTRO-ESOPHAGEAL JUNCTION

Adenocarcinomas of the gastro-esophageal junction (GEJ) are typically distinguished as adenocarcinomas of the esophagus (Barrett-carcinomas) and adenocarcinomas of the gastric cardia. In contrast to adenocarcinomas of the more distal stomach, the incidence of adenocarcinomas of the esophagus and gastric cardia has markedly increased in the last few decades, for reasons that are unclear (135-137). Furthermore, adenocarcinomas of the esophagus and gastric cardia share other epidemiological and clinicopathological characteristics (135). Whether all tumors arising at the GEJ should be regarded as one entity is still a topic of dispute. The etiology and pathogenesis of GEJ adenocarcinomas has remained largely unclear but Barrett’s esophagus, characterized by columnar epithelial metaplasia, due to chronic injury from gastroesophageal reflux is an identified predisposing condition (138). Barrett’s esophagus can subsequently progress to low-grade and high-grade dysplasia and eventually to invasive cancer (139). Furthermore, also intestinal metaplasia of the gastric cardia has been recognized (140). Besides reflux disease, other risk factors for GEJ cancer include smoking, increased fat intake and obesity (141-143). In contrast with the etiological factors involved in ‘non cardia’ gastric cancer, there is no consistent association with diet (135). Interestingly, infection with \textit{H. pylori} seems to protect against gastro-esophageal reflux, Barrett’s esophagus and GEJ-cancer, whereas it is recognized as a major risk factor for cancer of the more distal stomach (135,144,145).
Histopathologically the vast majority of cancers arising at the GEJ are adenocarcinomas. Signet-ring cell carcinomas are much less common in the proximal than in the distal stomach. Of the lesser common forms of cancer at the GEJ, adenosquamous carcinoma is the one most likely to be encountered (135).

**Molecular alterations in GEJ-carcinomas**

Several oncogenes and tumor suppressor genes are frequently involved in GEJ carcinomas (146). The c-ERBB2 (HER2/neu) proto-oncogene is overexpressed in 10-70% of esophageal adenocarcinomas. Overexpression of c-ERBB2 appears to be correlated with advanced tumor stage and metastasis (147). Activation of the RAS proto-oncogenes seems to be of little importance in Barrett’s adenocarcinomas and adenocarcinomas of the gastric cardia. Amplification of cyclin D1 is observed in 22%-64% of GEJ-cancer and appears to be a relatively early event (148). Furthermore, enhanced expression of acidic fibroblast growth factor (aFGF), c-MYC and c-SRC have been described in adenocarcinomas of the esophagus (146). Frequent allelic loss has been observed at chromosomes 3p, 5q, 9p, 9q, 13q, 17p and 18q both in adenocarcinomas in Barrett’s esophagus as well as in adenocarcinomas of the gastric cardia (149-151). The best-characterized somatic alteration found in tumors of this region are mutations of TP53, which are present in up to 60% of carcinomas of the gastroesophageal junction (135). Comparative genomic hybridization (CGH) has been used to compare tumors of the gastric cardia and Barrett carcinomas. Common altered regions included 3p14, 4q, 5q14-21, 9p21, 14q31-32.1, 16q23, 18q21 and 21q21. The distribution of these imbalances was similar in both groups. However, loss of 14q31-32.1 was significantly more frequent in Barrett-related adenocarcinomas when compared to cardia carcinomas (152-154). Microsatellite instability has been reported but true MSI-High (i.e. instability at >40% of the tested markers) appears to be infrequent in both Barrett carcinomas as well as in cardia carcinomas (151,155,156). Overexpression of COX-2 appears to be a frequent relatively early event in carcinogenesis of tumors at the GEJ, especially in Barrett carcinomas. Interestingly, the expression of COX-2 appears to be inducible by bile acids, both in cell-culture models as well as in cultured human biopsies from the esophagus (157,158). See chapter 6 for a more detailed introduction on the role of COX-2 in carcinogenesis of the esophagus.

**REFERENCES**


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OUTLINE OF THE THESIS
As mentioned earlier, cancer is the endstage of a multistep carcinogenic process during which many molecular alterations have accumulated. A better understanding of the molecular basis of cancer will ultimately be beneficial for patients and individuals at risk. The studies in this thesis focus on some molecular alterations frequently occurring in carcinomas of the stomach and of the esophagus.

Epstein-Barr virus (EBV) and gastric carcinogenesis:
Remote partial gastrectomy has been recognized as a major risk factor for gastric cancer. It was postulated that gastric stump cancer could provide a suitable model to study the molecular genetics of gastric carcinogenesis in general. In chapters 2 and 3 a comparison is made between ‘conventional’ gastric cancer and gastric stump cancer with respect to prevalence of H. pylori (HP) and Epstein-Barr virus (EBV) and molecular alterations of the p53 tumor suppressor pathway and the K-RAS proto-oncogene.

Chapters 4 and 5 elaborate on the role of EBV in gastric carcinogenesis. In chapter 4 EBV-positive and EBV-negative tumors are compared with respect to alterations at several tumor suppressor loci important in gastric cancer. In chapter 5 the ‘timing’ of EBV infection of gastric epithelial cells during gastric carcinogenesis is investigated.

Cyclooxygenase-2 (COX-2) expression in gastric and esophageal cancer:
Chapters 6 - 10 focus on the role of cyclooxygenase-2 (COX-2) in carcinogenesis of gastric and esophageal cancer. In chapter 6 the literature is reviewed with regard to the role of COX-2 in tumors of the gastrointestinal tract. Chapters 7 and 8 describe the expression of COX-2 in gastric carcinomas of different histological types and in precursor lesions of gastric cancer. Prostaglandin E-synthase is an enzyme downstream of COX-2 in the prostanoid synthesis pathway. In chapter 9 the expression of the microsomal isoform of this enzyme (mPGES) is described in gastric adenocarcinomas and gastric cancer cell lines. In chapter 10 the prognostic value of COX-2 expression in adenocarcinomas of the esophagus is evaluated.

Clinical applications of molecular biological tools:
Chapters 11 and 12 are case-reports dealing with the use of molecular techniques in diagnosis and the investigation of pathogenesis of individual tumors. Chapter 11 describes a case of a patient with seven tumors of the aerodigestive tract. In this case several molecular techniques are deployed to test whether the individual tumors are either metastases of a previous one or independent primary tumors. Chapter 12 deals with a rare adenosquamous carcinoma at the gastroesophageal junction. In this case the molecular techniques are used to test whether the two distinct histological components share the same molecular make-up and thus in fact comprise one tumor or whether it concerns a so-called collision tumor consisting of two pathogenetically independent tumors.

Chapters 13 and 14 provide a summary and concluding remarks in English and in Dutch.