Chapter 1

INTRODUCTION

1.1. ANATOMY OF THE STOMACH

The stomach is an extended section of the digestive tract between the esophagus and small intestine. The esophagus opens into stomach through cardiac orifice which is guarded by esophageal sphincter. The stomach can be divided into five regions the cardia, the fundus, the corpus/body, the antrum, and the pylorus (Figure 1). The stomach wall is composed of four layers: mucosa, sub mucosa, muscularis mucosa and serosa

![Figure 1: Anatomy of the stomach](image)

**Cancer of the stomach**

Gastric cancer can be of different types depending on the cell type from which it originates. Gastric adenocarcinoma which arises in the glandular epithelium of the gastric mucosa is known to be the more frequent type of cancer of the stomach followed by rarely occurring sarcomas and stromal tumors\(^1\). It is often diagnosed at an advanced stage leaving limited treatment options. At
present, surgical intervention remains to be the standard mode of treatment of patients with gastric cancer, while chemotherapy remains largely palliative. There is an increasing incidence of gastric cancer in many regions across the world and diagnosis and prognosis of gastric cancer still remains challenging.

1.2. EPIDEMIOLOGY AND RISK FACTORS

1.2.1. Epidemiology

Gastric cancer is the fourth most common malignancy and the second leading cause of cancer deaths in both males and females. Around 989,000 new cases of gastric cancer were estimated to have occurred in 2008. About 738,000 mortalities from gastric cancer were reported in the same year. More than 70% of cases are reported to occur in developing countries (Figure 2). Figure 3 shows the worldwide incidence and mortality rates for stomach cancer in both males and females. The highest incidence and mortality rate of gastric cancer are reported from Eastern Asia. In India, gastric cancer occurs more frequently in eastern and southern regions (Figure 4).

Figure 2: Incidence and mortality rate of the top ten cancers
Figure 3: Worldwide incidence and mortality rate of gastric cancer
Figure 4a: Nationwide incidence and mortality rate of gastric cancer in males

Figure 4b: Nationwide incidence and mortality rate of gastric cancer in females
1.2.2. Risk factors

Several risk factors are associated with the development of gastric cancer. The risk factors could be broadly classified into i) precursor conditions which might lead to the development of cancer and ii) other genetic and environmental factors that might predispose to the risk of developing cancer. These are discussed in detail below.

i). Precursor conditions

Gastric cancer has been reported to arise in the background of certain pathological conditions. Chronic atrophic gastritis and its associated abnormality intestinal metaplasia are often known to be precursors for gastric cancer. Atrophic gastritis is an inflammatory condition which is characterized by the loss of glandular cells and subsequently replaced by intestinal and fibrous tissues. It normally begins as a multifocus process in the distal stomach. Subsequently, there is a reduced gastric acid production when the foci coalesce leading to intestinal metaplasia followed by dysplasia and then carcinoma. Intestinal metaplasia has been associated with intestinal type cancers more frequently than diffused type carcinomas. However, epidemiologic studies have shown equal prevalence of diffused type carcinomas with intestinal metaplasia. It has been shown that atrophic gastritis and intestinal metaplasia are more prevalent in regions where there is high prevalence of gastric cancer. This in part is due to the common risk factors associated with the development of these conditions. It has also been reported that not all cases with these precursor conditions develop gastric cancer implying that neither atrophic gastritis nor intestinal metaplasia alone could lead to the development of gastric cancer.

Pernicious anemia

It has been shown that pernicious anemia is associated with a 2 to 3-fold increased risk of gastric cancer. Patients with pernicious anemia have been reported with prolonged acid suppression, hypergastrinemia and neuroendocrine hyperplasia which subsequently results in intestinal type gastric cancer and gastric carcinoids.

Gastric ulcers

Although, it was believed that gastric ulcers might lead to gastric cancer, epidemiological studies were not able to determine a clear association. However, patients undergoing partial
gastrectomy for benign disorders such as peptic ulcer disease have been shown to be associated with the risk of developing gastric cancer post surgery. \textit{H. pylori} have been discovered as the causative agent for peptic ulcers and lack of association between gastric cancer and peptic ulcer disease, suggests that the association of \textit{H. pylori} infection with gastric cancer is independent of the link between the infection and ulcer disease. \textit{H. pylori} infection is one of the well known risk factors associated with gastric adenocarcinoma. Infection with \textit{H. pylori} was strongly associated with progression of gastric adenocarcinoma in different populations around the world. Through prospective studies, it has been shown that patients with \textit{H. pylori} infection have three to six fold increased risk of gastric cancer as compared to uninfected individuals. Intestinal type cancers and cancers of the distal stomach are often associated with \textit{H. Pylori} infection. The precise role of \textit{H. pylori} infection in gastric carcinogenesis remains unclear, although it is associated with the development of chronic atrophic gastritis. However, gastric carcinoma develops in only a small proportion of infected patients, suggesting that genetic or environmental cofactors are required. The reports on Indian population depicts a different scenario, which shows \textit{H. pylori} infection may not play a significant role in cancer progression.

Other precursor conditions that are potential risk factors include Menetrier’s disease, gastric polyps, and adenomas.

\textbf{ii). Genetic and environmental factors}

\textbf{Family history of gastric cancer}

Hereditary type of gastric cancer was first discovered in 1800s when different individuals from Napoleon Bonaparte family were affected with the disease. It accounts for 1-3% of the total incidence of gastric cancers. Hereditary diffuse gastric cancer (HDGC) is known to be caused by the occurrence of germline mutations in the gene \textit{CDH1} encoding E-cadherin, a molecule which is essential for maintaining epithelial cell integrity. Around 40% of the HDGC cases were known to harbor \textit{CDH1} mutations while the genetic cause of others being unknown. Germline mutations in the gene \textit{TP53} which occurs in Li-Fraumeni syndrome also serves a predisposing factor for hereditary gastric cancer. Apart from familial breast cancer \textit{BRCA2} mutations are also known to be associated with hereditary form of gastric cancer. High frequency of extra colonic carcinomas which includes gastric carcinoma has been observed in a
proportion of families affected with hereditary nonpolyposis colorectal cancer (HNPCC)\textsuperscript{28}. Persons with blood type A, have been shown to possess increased risk of gastric cancer, especially for diffuse lesions \textsuperscript{7}.

**Diet**

Dietary pattern has been reported as one of the important risk factors for gastric cancer. Consumption of salted, smoked, or poorly preserved foods is one of the reported dietary factors for gastric cancer. Association of salt in the etiology of gastric cancer was discovered in the early 1960s. It was hypothesized that frequent intake of salt in large quantities leads to early atrophic gastritis, which in turn increases the risk of gastric cancer \textsuperscript{29}. Long-term ingestion of nitrates present in dried, smoked and salted foods seems to be associated with an elevated risk \textsuperscript{30-32}. Bacteria, which are exogenously introduced by consumption of contaminated food, are thought to convert nitrates into nitrites (free radicals), which are carcinogenic \textsuperscript{33,34}. Low consumption of fruits and vegetables in the diet has also been associated with gastric cancer \textsuperscript{35,36}. Epidemiologic studies have shown that consumption of fresh fruits and vegetables, or contained micronutrients, has a protective role against gastric cancer \textsuperscript{37}. Micronutrients such as vitamins C (ascorbate) and E (alpha-tocopherol), carotenoids (especially beta carotene), and selenium are associated with the possible role of prevention against gastric cancer \textsuperscript{31}. Smoking \textsuperscript{38} and alcohol consumption \textsuperscript{39} were also associated with an increased risk of gastric cancer.

1.3. PATHOLOGY OF GASTRIC ADENOCARCINOMA

Gastric adenocarcinoma arises from the glandular epithelium (mucosa) of the stomach. More than 90 percent of gastric cancers have been reported to be adenocarcinomas with the remainder being non-Hodgkin’s lymphomas, leiomyosarcomas, gastrointestinal stromal tumors and carcinoid tumors\textsuperscript{1}. Several classifications of gastric cancer have been proposed in the past decades: e.g. the classifications by WHO, Ming, Mulligan, and Laurèn, and Goseki \textit{et al}\textsuperscript{40}. Among all of the above classifications, Lauren’s was more widely and successfully used in the clinic. According to Lauren’s classification, adenocarcinomas are divided into two main histological types – intestinal and diffuse. The intestinal type characterized by cohesive neoplastic cells forming gland like tubular structures while the diffuse type is characterized with
a thickening of the stomach wall without a discrete mass \(^{41}\). Both these subtypes vary in their epidemiology, histopathology and in their pathogenesis.

**1.3.1. Intestinal-type adenocarcinomas**

Intestinal-type gastric adenocarcinoma is known to occur most frequently in elderly men, and is associated with relatively better survival. IGCA has been known to be resulted from a series of events triggered by etiological factors. Correa \textit{et al}\(^{42}\) has defined the stages of precancerous cascade as following: Intestinal-type carcinomas are preceded by a series of lesions appearing sequentially as the carcinogenic process advances. The stages of this precancerous cascade are well defined: normal cells progresses to chronic active non atrophic gastritis which then becomes multifocal atrophic gastritis followed by intestinal metaplasia (complete, then incomplete) later dysplasia and then invasive carcinoma \(^{5,42}\). The above mentioned cascade of events in a mouse model was studied by Fox \textit{et al}\(^{43}\) which is represented in Figure 5. Chronic, active non atrophic gastritis is represented by interstitial infiltration of the gastric mucosa by chronic inflammatory cells: lymphocytes, plasma cells, and macrophages. Subsequently, loss of gastric glands results in atrophy. Atrophic glands eventually attain intestinal phenotype characterized by absorptive enterocytes with a prominent brush border alternating with well-developed mucous goblet cells and sometimes Paneth cells (metaplastic). The next stage in the cascade, the metaplastic cells become neoplastic characterized by cellular crowding, large, hyperchromatic nuclei, and increase in the mitotic activity. These cells are termed dysplastic as long as they remain within the basement membrane. Once they cross the basement membrane, the cells become invasive \(^{42,44}\).
1.3.2. Diffuse-type adenocarcinomas

The precancerous cascade does not precede most diffuse-type gastric carcinomas (DGCA). No clear precancerous lesions have been reported for sporadic diffuse carcinomas. They are characterized by thickening of the gastric wall due to tumor infiltration leading to ‘linitis plastica’ (stomach being transformed to a rigid tube). DGCA cells are round and individually invade the gastric wall. Due to overproduction of mucin, the nuclei are pushed towards the cell membrane resulting in a characteristic signet-ring appearance (Figure 6). The pattern of invasion of the diffuse-type adenocarcinoma results from the absence of cohesion among tumor cells because of lack of intercellular adhesion molecules. Diffuse-type adenocarcinomas are reported to occur more frequently in women and individuals < age 50. It has also been shown to have a less favorable prognosis.

Figure 5: Histological progression of intestinal type gastric

Early gastric cancer is another subtype which does not fall into either diffused or intestinal type. It is known to be confined to the mucosa or sub mucosa, irrespective of the presence of regional lymph node metastasis. Early gastric cancer has been known to occur in approximately 50% of gastric cancers in Japan, while only 5-10% in western countries \(^{46}\).
1.4. Review of literature

Molecular alterations in gastric cancer

Over the past decade, a number of studies have been carried out in gastric cancers to identify molecular markers that are useful in diagnosis and prognosis of the disease. Changes at the molecular level could be sporadic or hereditary in nature. Some of the hereditary factors have been discussed in the previous section. This section is mainly focused on the changes at the molecular level in gastric cancer. The different types of changes that are characterized includes

i) Alterations in the genome
ii) Alterations in the transcriptome
iii) Alterations in the proteome
iv) Other molecular alterations

Gene expression changes

Genomic modifications in gastric cancer

Modifications at the genome level could be restricted to single base, which could lead to point mutations or it could be a short stretch which includes amplifications and deletions. Major anomalies that are observed at the genome level pertains to loss or gain of bigger regions in the genome including chromosomes or it could be genomic instabilities arising from defects in DNA synthesis. Some of the above mentioned changes that have been reported in gastric cancer are discussed below. Mutations in beta catenin have been found to occur at varying frequencies in gastric carcinomas. Germline mutations in E-cadherin have been reported in 50% of diffuse-type carcinomas. Other somatic mutations observed in this gene include in-frame deletions and point mutations. Gene copy number variations are frequently observed in gastric carcinoma. MET is a proto-oncogene that has been shown to be amplified in gastric cancer leading to aberrant expression. It is a tyrosine kinase receptor and has also been reported to be hyperphosphorylated leading to aberrant signaling in gastric cancer. ERBB2 is another tyrosine kinase receptor which is frequently amplified in gastric cancers.

Microsatellite instability (MSI) primarily occurs due to errors during DNA replication. This phenomenon is frequently observed in many tumors including gastric cancer. MSI has been reported to be prevalent in 2-22% of the gastric carcinomas. Hypermethylation of genes involved in DNA repair mechanism, hMLH1 and hMSH2 has been shown to have correlation with MSI.
phenotype \(^{47,53}\). Loss of heterozygosity (LOH) resulting in the inactivation of tumor suppressor genes namely p53, APC (Adenomatous polyposis coli) and DCC (Deleted in colon cancer) has been observed in gastric cancer \(^{54,55}\). It has been reported that around 30\% of gastric cancers harbor mutations or LOH at the p53 and 60\% of intestinal type cancers harbor a mutation/LOH of the APC gene \(^{55}\).

### Gene expression changes in gastric cancer

Expression of genes from genome is a highly regulated phenomenon. Any deviation from the regular expression causes the cells to function abnormally. Expression of genes could be monitored both at the level of transcription (mRNA level) and at the level of translation (protein). In this section, a summary of studies on gastric cancer at transcriptomic and proteomic level is provided

### Transcriptomic studies on gastric cancer

Due to aberrations in the genome, the cancer cells function abnormally. This can be detected by studying the expression of genes in cancer cells in comparison to the normal cells. Identifying differential expression of genes would lead to better understating of the tumorigenesis. It would also lead to identification of several molecular markers that might be useful in disease diagnosis, prognosis and therapy. This has been the goal of a large number of studies that has been carried out till date. In the past, studies were focused on looking at the expression of few genes chosen based on prior knowledge about those genes. For instance, aberrant expression of ERBB2 was tested in stomach tumors since it was known to be overexpressed in adenocarcinomas of colon, salivary gland and breast \(^{56}\). Overexpression of MET oncogene \(^{57}\) in gastric cancer was identified in a similar manner. However, with the advancement in technologies, unbiased approaches were employed to identify and quantify gene expression. Commonly used approaches to study gene expression include Northern blots, differential display, cDNA libraries, and Serial Analysis of Gene Expression (SAGE) \(^{58}\). Fushida et al. carried out Northern blotting to study the expression of HGF (Hepatocyte Growth Factor) and its receptor in a panel of gastric cancer cell lines \(^{59}\), cDNA libraries was used by Iwase et al. to identify overexpressed tyrosine kinases \(^{60}\). Differential display method was employed by Yoshie et al. to identify genes that were downregulated in
gastric cancer. SAGE was used by different groups which led to the identification of markers - S100A6, PLUNC and claudins that were differentially expressed in gastric cancer.

Discovery of microarrays revolutionized the study of gene expression, instead of looking at few genes at a time, now one could study the expression of thousands of genes simultaneously in a relatively simpler fashion. cDNA microarrays and oligonucleotide arrays were two major types of microarray platforms. In the following section, studies on gastric cancer using microarrays have been discussed.

**Microarray-based studies on gastric cancer**

Understanding the molecular alterations behind initiation and progression of gastric carcinogenesis is crucial in finding novel therapeutic and clinical targets. Specific gene expression profiles which correlate with histology, invasiveness, and survival have been identified in gastric cancer. Table 1 summarizes the studies carried out on gastric cancer using DNA microarrays.

Several expression profiling studies were done to distinguish cancerous and non-cancerous tissues based on the microarray profile. A cDNA microarray was used to analyze differences in gene expression between gastric cancer cell lines and normal cell lines, which found that SKB1, NT5C3, ZNF9, p30, CDC20, and FEN1, were significantly upregulated in gastric cell lines. A high density oligonucleotide microarray has been used to distinguish cancerous and noncancerous tissues which led to the identification of 162 genes that were highly expressed in gastric cancers with functions related to cell cycle, growth factor, cell motility, cell adhesion and matrix remodeling. Around 129 genes were found to be highly expressed in non-cancerous tissues with functions related to gastrointestinal-specific function and immune response. In another study using cDNA microarrays, significant overexpression of the S100A4, CDK4, MMP1, and b-catenin genes and downregulation of GIF gene was reported in gastric adenocarcinomas.

Notable number of gene expression studies has been done on different clinical subtypes of gastric cancers to study the correlation between the molecular profile and clinical behavior. In an
attempt to distinguish intestinal and diffused type adenocarcinomas, based on expression profile Lee et al. identified the enhanced expression of Alpha-II spectrin, Na/K-ATPase and KIAA0111 in intestinal type and enhanced expression of platelet-endothelial tetraspan antigen 3 in diffused type carcinoma. By comparing the expression profile of both the types distinct molecular signatures have been identified. Diffused type exhibited altered expression of genes related to cell-matrix interaction and extracellular matrix (ECM) components, whereas intestinal-type cancer represented genes related to enhancement of cell growth. Norsett et al. reported that BRCA2 could be used to differentiate intestinal from diffused type due to its over expression in intestinal type tumors.

Few gene expression studies have been carried out using samples from different clinical stages of tumorigenesis to classify them based on molecular profiles. cDNA microarray combined with laser captured microdissection (LCM) has been done to compare the gene expression profiles between primary gastric cancer and lymph node metastasis, which reported possible role of the following genes OPMCL, RNASE1, YES1 and ACK1 in tumorigenesis and metastasis of gastric cancer. In another study, cDNA microarray was used to study the differential expression between primary gastric adenocarcinomas, lymph node metastases and non-neoplastic adjacent gastric mucosa. Expression of several genes was found to be significantly associated with patient’s survival. In particular, high level expression of IGF-2 was associated with poor patient survival.

Few studies have applied statistical modeling based on gene expression profiles to predict tumor behavior with respect to tumor progression, metastatic potential, tumor recurrence and overall prognosis. Expression signatures from human gastric cancer tissues representing normal gastric mucosa, gastritis, intestinal metaplasia and adenocarcinoma were used to create molecular classifiers for these clinical subtypes. Based on the gene expression studies, three new molecular subtypes tumorigenic, reactive, and gastric-like were identified for gastric carcinoma which correlated with the clinical outcome. For instance, patients with gastric-like tumors have shown to exhibit better survival than patients belonging to the other two groups.
Studies have been carried out to observe if there are differences at molecular level due to geographical variations. Recently, Junilla and colleagues carried out a genome-wide expression analysis of gastric cancer tissues in Finnish and Japanese patients using cDNA and oligonucleotide microarrays. About 3,000 genes were found to be differentially expressed in the Finnish patients while Japanese patients showed differential expression in 149 genes.\(^7\)

Over the period of time some of the markers identified by these high-throughput studies were tested for its clinical use. Few of the markers were found to be useful in therapy and they are used in combination with chemotherapy. Monoclonal antibodies against EGFR, and tyrosine kinase inhibitors against VEGF have been tested in clinical trials in combination with chemotherapy.\(^9\) In spite of the advances in discovery and use of these markers, gastric cancer remains to be challenging and difficult to diagnose. Due to the lack of specific therapeutic targets, cytotoxic therapy remains the standard mode of treatment for unresectable gastric cancer patients and as adjuvant treatment for operable cases. This emphasizes the need for more studies at the molecular level to discover suitable biomarkers for diagnosis, prognosis and therapy.

### Table 1: Microarray studies on gastric cancer

<table>
<thead>
<tr>
<th>Samples</th>
<th>Significance</th>
<th>Experimental platform</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twenty tumors</td>
<td>Identified 61 genes that were upregulated and 63 genes that were downregulated in tumors</td>
<td>cDNA microarrays</td>
<td>73</td>
</tr>
<tr>
<td>Cell lines one from primary tumor and five metastatic lines</td>
<td>Twenty four genes including (CD44), keratins 7,8,14, (ALDH1A) were upregulated and 17 genes including IL2RG and ITGB4 were downregulated in primary gastric cancer as compared to metastatic cells</td>
<td>cDNA microarrays</td>
<td>92</td>
</tr>
<tr>
<td>Ninety tumor tissues and twenty two normal tissues</td>
<td>Identified PLA2G2A as a prognostic marker for long term survival of gastric cancer patients</td>
<td>cDNA microarrays</td>
<td>76</td>
</tr>
<tr>
<td>Twelve gastric carcinoma cell lines and 15 cell lines from lymphoid, endothelial, stromal and other epithelial cancers</td>
<td>Development of a resource of gene expression profiles of gastric cancer cell lines</td>
<td>cDNA microarrays</td>
<td>72</td>
</tr>
<tr>
<td>SNU1, primary and metastatic KATO-III, SNU-5, SNU-719, MKN45P, HS39 cell lines</td>
<td>Upregulation of CEA, 14-3-3, CD44, Ubiquitin A and several ESTs in gastric cancer</td>
<td>cDNA microarrays</td>
<td>95</td>
</tr>
<tr>
<td>Forty three tumors and adjacent gastric mucosa</td>
<td>78 gene scoring system has been identified to predict prognosis</td>
<td>cDNA microarrays</td>
<td>78</td>
</tr>
<tr>
<td>Five primary gastric cases</td>
<td>Cell cycle related genes and growth factors were found to be overexpressed in metastatic cells</td>
<td>cDNA microarrays</td>
<td>79</td>
</tr>
<tr>
<td>Ten paired tumor and normal tissues</td>
<td>A supervised learning classification approach was used to identify predictors of early and late gastric cancers</td>
<td>cDNA microarrays</td>
<td>80</td>
</tr>
<tr>
<td>Twenty two paired tumor and normal tissues</td>
<td>78 genes were upregulated in 398 were downregulated in tumors</td>
<td>cDNA microarrays</td>
<td>70</td>
</tr>
<tr>
<td>Ninety primary gastric cancers, 14 metastatic and 20 non-neoplastic tissues</td>
<td>Identification of gene expression signatures that correlated with patients survival</td>
<td>cDNA microarrays</td>
<td>69</td>
</tr>
<tr>
<td>Thirty five gastric tumors</td>
<td>A subset of genes related to chemo resistance in gastric tumors were detected in this study</td>
<td>Oligonucleotide arrays</td>
<td>81</td>
</tr>
<tr>
<td>paired tissues from 124 patients</td>
<td>Molecular signatures for chronic gastritis and Intestinal metaplasia was identified</td>
<td>cDNA microarrays</td>
<td>68</td>
</tr>
<tr>
<td>gastric cancer cell lines</td>
<td>inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) as a target for peritoneal dissemination of gastric cancers</td>
<td>cDNA microarrays</td>
<td>90</td>
</tr>
<tr>
<td>RF-1 and RF-48 cell lines</td>
<td>P18(INK4C) was found to be downregulated in metastatic cell line as compared to primary tumor cell line</td>
<td>cDNA microarrays</td>
<td>88</td>
</tr>
<tr>
<td>RF-1 and RF-48 cell lines</td>
<td>TSC403, GBP1, TK2, PP2A, PP2R5B and AHR were found to be downregulated due to inactivation by DNA methylation.</td>
<td>cDNA microarrays</td>
<td>95</td>
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<tr>
<td>Five paired gastric tumors</td>
<td>SPARC was found to be highly expressed in intestinal as well as diffused type cancers</td>
<td>cDNA microarrays</td>
<td>89</td>
</tr>
<tr>
<td>Thirty paired tumor and normal tissues</td>
<td>A novel gene, MDSCBC11 clone was found to be significantly downregulated</td>
<td>cDNA microarrays</td>
<td>82</td>
</tr>
<tr>
<td>Study Description</td>
<td>Findings</td>
<td>Method(s)</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
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<tr>
<td>Gastric adenocarcinoma and non cancerous precursors (normal gastric mucosa, gastritis mucosa and intestinal metaplasia)</td>
<td>Attempted to generate molecular classifiers to distinguish cancer vs. non cancerous conditions</td>
<td>cDNA microarrays</td>
<td>91</td>
</tr>
<tr>
<td>Twenty paired diffused type tissues</td>
<td>LCM derived cells from diffused tumors were compared with normal mucosa. Genes related to cell matrix adhesion and extracellular matrix were found to be differentially regulated</td>
<td>cDNA microarrays</td>
<td>72</td>
</tr>
<tr>
<td>Seventeen tumors vs. universal control</td>
<td>BRCA1 was found to be overexpressed in intestinal type tumors</td>
<td>cDNA microarrays</td>
<td>92</td>
</tr>
<tr>
<td>Gastric cancer cell lines</td>
<td>SKB1, CDC20, and FEN1 genes were found to be overexpressed and MT2A and CXX1 genes were found to be downregulated in gastric cancer cells</td>
<td>cDNA microarrays</td>
<td>85</td>
</tr>
<tr>
<td>Twelve paired tumor tissues</td>
<td>By carrying out aCGH and microarray analysis, it was shown that GRB7 and HER-2 displayed highest correlation between DNA copy number changes and gene expression profiles</td>
<td>cDNA microarrays</td>
<td>84</td>
</tr>
<tr>
<td>Twenty two primary tumors and 8 noncancerous tissues</td>
<td>In cancer tissues, 162 genes were found to be upregulated and 169 genes were found to be downregulated as compared to normals</td>
<td>Oligonucleotide arrays</td>
<td>67</td>
</tr>
<tr>
<td>Eight normal tissues and 38 primary tumors</td>
<td>ERBB2, MUC1, GRB7, PPP1R1B and PPARBP has shown both copy number variation and aberrant expression</td>
<td>Oligonucleotide arrays</td>
<td>83</td>
</tr>
</tbody>
</table>

**Proteomic alterations in gastric cancer**

Genomic and transcriptomic changes reflect in changes in the proteome. Proteins are the true workhorses in a cell. Hence, proteins are considered as suitable markers to monitor the condition of tissues and organs. Any abnormality in the function of cells could be detected by looking into
the protein profiles. Protein markers are one of the major thrust in the field of biomarkers. In
gastric cancer, several studies have been carried out to identify deregulated or defective proteins
contributes to tumorigenesis and its progression. In the past decade, due to limitations in
technology, the proteomic studies were largely limited to the study of a single protein or few
proteins in a given disease condition. For instance, number of biochemical and
immunohistochemical studies was done looking at the expression of individual proteins in gastric
cancers. This approach was proven useful to validate biomarkers. However, it was not possible to
study the entire proteome in an unbiased manner. Discovery of mass spectrometry eliminated this
barrier leading to a paradigm shift in the field of proteomics. Since then, many studies on gastric
cancer have been done using mass spectrometry. An overview of these studies is provided below.

**Mass spectrometry-based studies on gastric cancer**

Currently, the field of proteomics is largely driven by mass spectrometry-based studies\(^99\). Various studies have been carried out on gastric cancer using cell lines, tissues and serum to
identify potential candidates. Table 2 summarizes the proteomic studies done till date on gastric
cancer. Two dimensional gel electrophoresis (2-DE) followed by MALDI-TOF MS/MS analysis
has been widely used to identify biomarkers. By this approach, many studies have been done
using tumor tissues and matched normal tissues to identify differentially expressed proteins in
gastric cancer\(^100-112\). These studies led to the identification of a few tumor specific markers
which includes annexin V, carbonic anhydrase, MAWBP, transgelin, HSP60 and desmin.
Recently, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry was employed
on endoscopic biopsy samples which led to the identification of alpha-defensin-1, alpha-
defensin-2, calgranulin A, and calgranulin B to be overexpressed in tumors\(^113\).

Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF), is
a variation of MALDI-TOF. In MALDI, the samples are mixed with a matrix and directly
subjected to ionization followed by detection using time of flight mass spectrometer (TOF). In
SELDI, the samples are applied to a chip or surface coated with different chromatographic
chemistries that allows for on chip separation of proteins, minimizing protein loss and reducing
the complexity of the sample\(^114\) followed by detection using TOF. It has been widely used to
analyze body fluids and tissue extracts to identify signature peaks pertaining to a specific disease
including cancers\textsuperscript{115}. Serum from gastric cancer and matched normal has been analyzed using SELDI-TOF by different groups\textsuperscript{116-120}. These studies have led to the identification of signature peaks specific to cancer. However, this method suffers from number of limitations\textsuperscript{121}. Some of the major limitations include lack of consistency in the data and lack of specificity and sensitivity. Recent improvements in mass spectrometric technology and the development of novel quantitative methods have led to the discovery of reliable methods to discover biomarkers.

Quantitative proteomic approaches that are currently in use include Stable Isotope labeling with amino acids in Cell culture (SILAC) which is an \textit{in vivo} labeling and Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) which is an \textit{in vitro} labeling method\textsuperscript{99}. Since these methods are relatively new, only a handful of studies have used these approaches to identify markers for gastric cancer. iTRAQ has been employed recently with respect to gastric cancer\textsuperscript{122-125}. Plasma samples collected from mice with gastric tumors were compared with plasma from healthy mice by iTRAQ. In this study, \textit{ITIH3} was found to be significantly overexpressed which has been validated using plasma samples from patients with gastric cancer\textsuperscript{125}. In another study, iTRAQ labeling coupled to LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) was used to compare early-stage and late-stage gastric cancer patients that reported C9 protein as a potential marker\textsuperscript{124}. Using tissue samples to discover potential biomarkers could be both advantageous and disadvantageous. Tissue specimen from patients or controls reflects the \textit{in vivo} system or the endogenous expression levels of proteins. However, due to its complexity, it is often found to be associated with other cell types that hinder analysis. It is often difficult to analyze expression of proteins specific to epithelial compartment. This could be circumvented by using cell lines. A significant portion of the synthesized proteins in cells are known to be secreted and all they are referred to as secretome. These proteins are of major interest in biomarker research as it could be easily detected in body fluids\textsuperscript{126}. Identification of these proteins specifically secreted by cancer cells could advance biomarker discovery. Using cell lines offers advantages to look into this class of proteins that are otherwise undetectable by analyzing tissues. Studies have been carried out in other cancers targeting the secreted component of the cells, and identifying the aberrantly expressed secreted proteins\textsuperscript{127-129}. Till date such an approach has not been taken in gastric cancer. Only recently, a study has been carried out by Yang \textit{et al} to identify secreted proteins in gastric cancer\textsuperscript{123}. This study was done using
iTRAQ-based tandem mass spectrometry to quantify proteins secreted by gastric cancer cell lines as compared to normal gastric epithelial cells. Through this study, cathepsin S was found to be overexpressed in tumor cells as compared to normal cells. SILAC-based strategy has been proven to be more suitable approach to study secretome of cancer cells. Till date there have not been any reports that employed in vivo labeling method to quantify secreted proteins from gastric cancer.

**Table 2: Mass spectrometry-based proteomic approaches to investigate gastric cancer**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Findings</th>
<th>Type of mass spectrometer</th>
<th>Citation</th>
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<tr>
<td>Serum</td>
<td>Identification of 71 signal clusters unique to gastric cancer</td>
<td>SELDI-TOF</td>
<td>116</td>
</tr>
<tr>
<td>Tissues</td>
<td>Elevated levels of 14-3-3 zeta, calceulin, keratin, apolipoprotein A-1 precursor, proteasome activator complex subunit, nucleoside diphosphate kinase, nicotinamide N-methyltransferase, and pyridoxal kinase identified in gastric cancer</td>
<td>MALDI-TOF</td>
<td>103</td>
</tr>
<tr>
<td>Serum</td>
<td>Nine proteins were overexpressed and seven proteins under-expressed</td>
<td>SELDI-TOF</td>
<td>119</td>
</tr>
<tr>
<td>Tissues</td>
<td>Increased cathepsin B serum levels are associated with advanced tumor stages and progressive disease. Also there was decreased expression of gastricscin/pepsinogen II.</td>
<td>MALDI-TOF</td>
<td>101</td>
</tr>
<tr>
<td>Cell line</td>
<td>Thirteen proteins differentially regulated</td>
<td>MALDI-TOF</td>
<td>111</td>
</tr>
<tr>
<td>Serum</td>
<td>Three protein peaks significantly different between GC patients and normal.</td>
<td>SELDI-TOF</td>
<td>117</td>
</tr>
<tr>
<td>Tissues</td>
<td>Protein expression of MnSOD and HMG-1 was demonstrated to be up-regulated in tumor tissues compared to in no tumor tissues. On the other hand, the CA I and II, FOV, AST and GST proteins were revealed to be down-regulated in tumor tissues.</td>
<td>Q-TOF</td>
<td>109</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>Cell line</td>
<td>Twenty three proteins were differentially regulated. HSP27, HSP60, Prx2 were found to be potential candidates</td>
<td>MALDI-TOF</td>
<td>112</td>
</tr>
<tr>
<td>Tissues</td>
<td>12 up-regulated and 13 down-regulated. Decreased Expression of MAWBP and MAWD Gene.</td>
<td>MALDI-TOF</td>
<td>110</td>
</tr>
<tr>
<td>Serum</td>
<td>6 most discriminating peaks between cancer and normal were identified.</td>
<td>SELDI-TOF</td>
<td>120</td>
</tr>
<tr>
<td>Tissues</td>
<td>Forty-two distinct proteins that were differentially expressed at least twofold between the tissues were identified. Transgelin protein was overexpressed.</td>
<td>MALDI-TOF</td>
<td>102</td>
</tr>
<tr>
<td>Tissues</td>
<td>Ten over-expressed and five under-expressed proteins in stomach cancer tissues compared with normal tissues.</td>
<td>MALDI-TOF</td>
<td>105</td>
</tr>
<tr>
<td>Cell line</td>
<td>iTRAQ analysis identified Sorcin as a biomarker</td>
<td>Q-TOF</td>
<td>122</td>
</tr>
<tr>
<td>Serum</td>
<td>Five protein peaks chosen as components of the best biomarker pattern for diagnosis of gastric cancer</td>
<td>SELDI-TOF</td>
<td>118</td>
</tr>
<tr>
<td>Tissues</td>
<td>Nine upregulated proteins and 20 downregulated.</td>
<td>MALDI-TOF</td>
<td>107</td>
</tr>
<tr>
<td>Tissues and serum</td>
<td>Human neutrophil peptides 1–3 (HNPs 1–3) and Macrophage migration inhibitory factor (MIF) found to be elevated in gastric cancer relative to adjacent normal mucosa</td>
<td>SELDI-TOF</td>
<td>106</td>
</tr>
</tbody>
</table>
Plasma | C9 protein found to be significantly upregulated | Q-TOF | 124  
---|---|---|---  
Tissues | Twenty-six proteins were upregulated and 6 proteins were down-regulated in tumor tissue compared to control. | MALDI-TOF | 100  
Tissues | Decreased expression of S100P in gastric cancer than in normal gastric mucosa. | LTQ-Ion Trap | 104  
Tissues | SBP1 protein was found to be downregulated in gastric cancer | MALDI-TOF | 108

**Other molecular alterations**

Other modifications at the molecular level includes, epigenetic changes (epigenomic alterations), RNA inhibited, in particular changes in miRNA expression and regulation. Epigenetics refers to inheritable changes in the genome that does not involve direct change in the DNA sequence. One of the most common epigenetic modification observed in cancers include promoter hypermethylation which leads to silencing of genes involved in tumor suppression. This results in inactivation of tumor suppressor genes. In gastric cancer, tumor suppressor genes that are found frequently to be inactive due to epigenetic modifications include *MLH1*, E-cadherin, *APC* and p16. Methylation studies in gastric cancer are evolving and more genes are being found to be methylated. However, the specificity of methylation events is still questionable and further studies are warranted to find genes silenced in gastric cancer. The other rapidly emerging field is the study of miRNAs and its role in cancers. miRNAs have been reported to play both oncogenic and tumor suppressor roles in cancers. miRNAs have been reported to play both oncogenic and tumor suppressor roles in cancers. It is shown that they exert their function by modulating the expression of the genes that they regulate. In gastric cancer deregulation of several miRNAs (miR-130b, miR-21, miR-15, miR-93, miR-9, miR-106a etc) have been identified to be associated with defects in apoptosis and promoting metastasis. Recently, circulating miRNAs have been discovered which has the potential to serve as biomarkers. miR-17-5p, miR-21, miR-106a and miR-106b has shown to be present at higher levels, whereas let-7a has shown to be present at lower levels in plasma from gastric cancer patients.