E-CADHERIN AND GASTRIC CANCER

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Despite a rapid decline in the incidence, gastric cancer still remains the second commonest cause of cancer mortality in the world. The prognosis has not been changed in the last few decades. The recent advancement in molecular biology may potentially offer better diagnostic and prognostic indications. In the dissertation, the changing epidemiology and molecular advances of gastric cancer have been reviewed. One of the most important adhesion molecules, E-cadherin, was chosen as an illustration of the recent studies in molecular markers. Its role in gastric carcinogenesis was discussed.

A prospective study investigating serum soluble E-cadherin as a prognostic marker was carried out. Concentrations of soluble E-cadherin from 116 patients with histologically confirmed gastric adenocarinoma and 40 healthy subjects were measured with immunoenzymometric method using a commercially available sandwich ELISA kit based on monoclonal antibodies.

Logarithm of means of soluble E-cadherin concentration was significantly higher in patients with gastric cancers (3.85 ± 0.28) than healthy subjects (3.71 ± 0.18) ($p = 0.001$), and in palliative/conservatively treated cancers (3.91 ± 0.35) than operable cancers (3.78 ± 0.19) ($p = 0.015$). The cut-off value calculated from discriminant analysis on the operability and inoperability/palliative treatment was 7025 ng/ml. Soluble E-cadherin concentration higher than this cut-off value predicts tumour (T4) depth invasion ($p = 0.020$, C.I. 1.008-1.668) and palliative/conservative treatment ($p = 0.023$, C.I. 1.038-2.514). The study shows that serum soluble E-cadherin is a potential valid prognostic marker for gastric cancer. A high concentration predicts palliative/conservative treatment and T4 invasion.

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Chapter 3: Soluble E-cadherin in gastric cancer

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Chapter 1

INTRODUCTION

1.1 Introduction

With the rapid decline in the global incidence of gastric cancer from the 1930s to the 1970s, the discovery of *Helicobacter pylori (H. pylori)*, and the advancement in molecular biology, the view towards gastric cancer has been changing. Nevertheless, gastric cancer remains the second commonest cancer in morbidity and mortality globally. This chapter reviewed the recent changes in the incidence and disease pattern, the concept of *H. pylori* as the etiological factor and the development of molecular biology.

1.2 Epidemiology and Aetiology of Gastric cancer

1.2.1 The changing Epidemiology

The gastric cancer incidence has declined rapidly over the recent few decades. The decline took place globally. (Haenszel et al, 1958; Munoz et al, 1971; Hirayama et al, 1975; Piper et al, 1978; Waterhouse et al, 1976) The cause for this decline in the incidence of gastric cancer is still a medical mystery and occurred before the discovery and eradication of *H. pylori.* The decline took place earliest in countries with low gastric cancer incidence such as the United States in 1930s, whereas the onset of decline in countries with high incidence like Japan was slower. In China, the decline was less dramatic than other countries. Zheng et al, (1993) reported that in Shanghai, despite an overall decrease in gastric cancer incidence, an increase have been observed in the oldest and the youngest group and less remarkable decline was observed among women than in men. The rise in incidence of gastric cancer among those at 25-34 years is note worthy since this may signal the introduction of new environmental factors and the age of onset of developing gastric cancer in Chinese population is younger than that in the Western.

The diffuse and intestinal types of gastric cancer as classified by Lauren (1965) described two biological entities that are different in regard to epidemiology, etiology, pathogenesis and behaviour. While there was a decline in the incidence of the intestinal type in the recent few decades worldwide as parallel to the overall incidence of gastric cancer, a gradual increase in the diffuse type was observed, which now accounts for approximately 30% of gastric carcinoma in some reported series (Ideda 1995).

An explosive increase in incidence of gastric cancers confined to the cardia has been observed (Dupont et al, 1978; Adashek et al, 1979; Kampschoer et al, 1989; Powell et al, 1990). The shift from distal to proximal stomach was partially contributed by the decrease in the distal cancers. The proximal tumours share demographic and pathological features with Barrett's associated oesophageal adenocarcinoma and are more likely to occur in men and this parallels the male predominance in the increasing incidence of lower third oesophageal carcinoma. The proximal tumours differ from distal tumours in that they are not associated with severe form of gastritis characterised by atrophy and/or intestinal metaplasia. They tend to be more aggressive than those arise from distal sites. It has been proposed that environmental factors or chemical carcinogens eg. cigarette and alcohol have been particularly associated with the cardiac carcinoma (Kalish et al, 1984). In fact, it has been proposed that carcinoma at the cardia is a different entity from that of the rest of the gastric carcinoma.

The incidence of gastric cancer varies with different geographic regions as well. High incidence is noted in Asian countries like Japan, Korea and China; part of Europe like Ireland; and the South American countries like Chile and Columbia. A difference in incidence and mortality from north to south has been observed in several countries, with the northern prefectures have higher mortality risk than those in the south. This gradient is particularly marked in the Northern Hemisphere (Wynder et al, 1963; Correa, 1982; Wong et al, 1998). Whereas in the Southern Hemisphere, the mortality risk tends to be higher in the southern parts (Correa et al, 1970; Cuello et al, 1976). It appears that higher geographic latitudes are associated with a higher gastric cancer risk.

1.2.2 Environmental Risk Factors and H. pylori

Risk of gastric cancer is associated with socioeconomic status. Subjects from lower socioeconomic class had approximately twice as high risk of developing intestinal type gastric cancer as subjects from higher socioeconomic group (Haenszel et al, 1958; Wynder et al, 1963; Berndt et al, 1968; Barker et al, 1990). On the contrary, proximal gastric cancers were associated with higher socioeconomic class (Powell et al, 1990).

Large epidemiology studies demonstrating the association between diet and gastric cancer were mainly based on the amount of food imported and produced rather than the actual food consumption (Howson et al, 1986). This takes no account of the losses during storage, distribution and consumption of food, nor any ethnic dietary differences. The association between N-nitroso compounds and gastric cancer has been summarised by Bartsch et al in 1987. The risk of gastric cancer induced by N-nitroso compounds has

been demonstrated in animal experiments (Magee et al, 1976; Drukrey et al, 1975; Bulay et al, 1979). An increase in gastric nitrite was observed in patients with intestinal metaplasia, dysplasia and gastric cancer (Ruddell et al, 1978; Jones et al, 1978; Stewart et al, 1967). The use of nitrate-based fertilizers (Jones et al, 1978; Schlag et al, 1980; Frazer et al, 1980) and pickled foods that contain nitrosated products (Haenszel et al, 1972; Sato et al, 1959) have been shown to positively correlate with gastric cancer. High salt intake has been shown to damage stomach mucosa and increase the susceptibility to carcinogenesis in rodents (Tatematsu et al, 1975; Takahashi et al, 1984; Hanawa et al, 1980). The positive correlation between nitrate intake, salt excretion and gastric cancer has recently been reported in the Intersalt study involving 24 countries from 39 populations (Joossens et al, 1996).

The World Health Organisation's International Agency for Research on Cancer has recently classified *H. pylori* as a Group 1 or definite carcinogen (IARC 1994). The etiological role of *H. pylori* on gastric cancer was based on Correa's model (1975, 1983, 1988): chronic atrophic gastritis to intestinal metaplasia, dysplasia and finally carcinoma. *H. pylori* has been shown to be strongly associated with gastric atrophy and intestinal metaplasia (Kikuchi et al, 1995; Parsonnet et al, 1991; Wong et al, 1999). Large case control and cohort studies have shown the relationship between *H. pylori* and adenocarcinoma (Parsonnet et al, 1991; Nomura et al, 1991; Talley et al, 1991; Forman et al, 1991, Eurogast study group, 1993) in both intestinal and diffuse types. *H. pylori* infection has been estimated to increase the risk of gastric cancer by sixfold (Forman et al, 1991). Tsugane et al. found that in a Japanese population, higher salt intake correlates

with higher prevalence of *H. pylori* infection (1994). It was postulated that gastric mucosal damage caused by high salt intake facilitated *H. pylori* infection. Gastric juice of *H. pylori*-positive individuals had lower concentration of vitamin C than *H. pylori*-negative individuals, but the concentration returned to normal when *H. pylori* was eradicated (Schorah et al, 1991). Therefore, vitamin C could play an important role in preventing the damage caused by *H. pylori* through its antioxidant effect (Schorah et al, 1991). Lower socioeconomic status was associated with higher prevalence of *H. pylori* (Graham et al, 1991). However, large interventional studies are needed to directly prove the causative role of *H. pylori* in gastric carcinogenesis (Forman et al, 1998).

Despite the proposal of dietary, environmental factors and the identification of *H. pylori*, the rapid global decline in gastric cancer is still not fully explainable. An interesting hypothesis has been proposed is the popularisation of refrigerators as a pivotal point for the decline (Coggon et al, 1989; La Vecchia et al, 1990). Refrigerators improved the storage of food, thereby reducing salting for preserving food and preventing bacterial and fungal contamination of food. Refrigeration also enables fresh food and vegetables more readily available which may be a valuable source of antioxidants important for cancer prevention.

1.3 Molecular Biology of Gastric Cancer

It has been well known that the transformation of a normal epithelial cell to a malignant cell results from the accumulation of multiple gene abnormalities. (Fearon and Vogolstein, 1990) As the gastric epithelium progresses from chronic gastritis to intestinal metaplasia, dysplasia and finally to carcinoma as in the Correa's model (1975, 1983, 1988), a progressive accumulation of molecular changes have been observed.

1.3.1 Precancerous lesions

Microsatellite instability (Strickler et al, 1994; Semba et al, 1996) and telomerase reactivation (Tahara et al, 1994) have been shown to be the earliest changes in gastric carcinogenesis. Microsatellite instability has been detected in 42% of gastric adenomas and 33% of intestinal metaplasia (Strickler et al., 1994 Semba et al., 1996). A shorter than normal telomere length was observed in intestinal metaplasia (Tahara et al., 1994). Furthermore, mutations of K-ras, adenomatous polyposis coli (APC), and p53 genes have also been detected in the precancerous lesions like gastric adenomas (Kihana et al, 1991; Tamura et al, 1994), chronic atrophic gastritis (Tahara et al, 1994; Tohdo et al, 1993), intestinal metaplasia and dysplasia (Shiao et al, 1994; Ochiai et al, 1996).

1.3.2 Advanced carcinoma

In addition to microsatellite instability and telomerase reactivation, mutations of P53 (Sano et al, 1991), K-ras (Nanus et al, 1990) and APC genes (Nakatsuru et al, 1992); amplification and overexpression of c-myc gene (Shibuya et al, 1985; Ciclitira et al, 1987) are progressively accumulated in the advanced gastric cancer. Further, cyclin D1

overexpression was found in around half of the gastric cancers (Moss et al, 1996). Amplification of the cyclin E gene to 3-10-fold was also found in gastric cancer tissues, particularly in advanced intestinal type (Akama et al, 1995), and its amplification correlated well with tumour staging, invasiveness and histological grading.

1.3.3 Tumour progression

Multiple autocrine/paracrine loops of tyrosine kinase receptors and peptide regulatory growth factors are involved in the progression of advance gastric tumours. The circulating level of the growth peptide is elevated while its receptor is amplified or overexpressed. These include hepatocyte growth factor (Taniguchi et al, 1997) and c-met gene (Kuniyasu et al, 1992); fibroblast growth factor and K-sam gene (Tahara, 1993); epidermal growth factor and c-erbB-2 and HER-2/neu genes (Akiyama et al, 1986; Coussens et al, 1985; Kameda et al, 1990; Uchino et al, 1993; Tal et al, 1988); EGF /TGFα and EGF receptors (Tahara, 1990). Other peptide regulatory factors expressed by gastric cancers include transforming growth factor (TGFα) (Naef et al, 1996), Cripto (Kuniyasu et al, 1991), platelet-derived growth factor, insulin-like growth factor II, basic fibroblast growth factor, IL-1, IL-6 and IL-8.

The elevation of the circulating peptide or the overexpression/amplification of the corresponding receptors may have prognostic importance. Elevated circulating level of hepatocyte growth factor was frequently found in patients with distant metastases (Taniguchi et al, 1997). An aberrant 6.0kb mRNA transcript of c-met gene that was expressed in 52% of gastric carcinoma tissues closely correlated with tumour staging,

lymph node metastasis and depth of invasion (Kuniyasu et al, 1993). Overexpression or amplification of c-erbB-2 gene is found to be associated with poor prognosis (Yonemura et al, 1991) and related to invasion and nodal involvement in well differentiated adenocarcinoma (Mizutani et al, 1993). Coexpression of EGF and TGFα in gastric cancer showed a greater degree of gastric wall invasion and lymph node metastasis (Yonemura et al, 1992; Yasui et al, 1988; Tokunaga et al, 1995). Cripto correlates well with the tumour stage and prognosis of gastric cancer (Kuniyasu et al., 1991).

1.3.4 Metastasis

A lot of growth factors, cytokines and adhesion molecules are involved in the complex process of metastasis. Unstable or reduced expression of one of the adhesion molecule, E-cadherin has been postulated to account for the invasive ability or metastatic potential. Decreased expression of E-cadherin, ranging from 17% (Shimoyama and Hirohashi, 1991) to 92% (Mayer et al, 1993), has been observed in gastric cancer. The better 3- and 5-year survival rates in E-cadherin positive tumours than E-cadherin negative tumours have been shown in a study of 413 gastric cancers (Gabbert et al, 1996). The decrease expression of E-cadherin could be due to genetic changes that have frequently been noted in scirrhous type or poorly differentiated gastric cancer (Oda et al, 1994; Yasui et al, 1995). On the other hand, the overexpression of the splice variant of CD44, another adhesion molecule, was found in all gastric cancer tissues and their metastatic foci (Yokozaki et al, 1994) and it significantly correlated with tumour recurrence and increased mortality in curatively resected patients (Mayer et al, 1993). A higher positive stain of matrix metalloproteinases (MMP-2 and MMP-9), gelatinases for degrading the

basement membrane, was found in patients with advance than early gastric cancer and in patients with poorly differentiated than well differentiated adenocarcinoma. (D'Errico et al, 1991).

1.3.5 Intestinal versus diffuse types of gastric carcinoma

Genetic differences have been observed in intestinal and diffuse type, suggesting two different pathways of carcinogenesis. Mutations of p53 gene are essentially restricted to the intestinal type in the early phase, but involved in both types in the advanced stage (Ranzani et al, 1995; Uchino et al, 1993). LOH and mutation of the APC (Nakatsuru et al, 1992) and the deleted in colon cancer (Uchino et al, 1992) genes, amplification of c-erb-2 gene (Tahara, 1993), overexpression of cyclin D1 (Moss et al, 1996) are frequently associated with intestinal type gastric cancers but seldom found in diffuse type.

On the other hand, microsatellite instability is found in 64% in diffuse type but only 17% in intestinal type (Han et al, 1993). Amplifications of c-met and k-sam tyrosine kinase receptor genes and overexpression of EGF family, TGFα, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-II) and fibroblast growth factor (FGF) (Tahara, 1990) are frequently found in diffuse type carcinomas (Tahara, 1993). The involvement of the cadherin gene, which has been called the invasion suppressor gene, seems to be one of the most important steps in diffuse type carcinogenesis and takes place at an early stage. Decreased expression of E-cadherin by immunohistochemical staining has been found in most of the diffuse type gastric cancer which is characterised by diffuse infiltration and high invasion potential.

1.3.6 Helicobacter pylori and the molecular changes

H. pylori has been postulated to be a potent carcinogen as evidenced by epidemiological and cohort studies. The relationship between *H. pylori* and the molecular changes may be another way to prove the role of *H. pylori* in gastric carcinogenesis. Activation of telomerase is responsible for cell immortality and is the most common fundamental event in gastrointestinal cancers. Kuniyasu et al (1997) found that the degree of *H. pylori* infection increased parallel with the level of human telomerase RNA expression (hTR) and telomerase positivity in 26 carcinoma tissues. However, microsatellite instability has been shown to be independent of *H. pylori* infection (Lin et al, 1995). Downregulation of the E-cadherin protein has been shown to be significantly associated with *H. pylori* infection in patients with normal gastric mucosa, gastritis, gastric ulcer and duodenal ulcer (Terres et al, 1998). Whether *H. pylori* infection is the cause for the downregulation of the E-cadherin protein and whether the downregulation subsequently leads to the cancerous process is still under study. Further molecular evidence is needed to establish *H. pylori* infection as the etiological factor for gastric carcinogenesis. Studies on whether eradication of *H. pylori* results in reversion or halting of the molecular events are important both in the understanding of gastric carcinogenesis as well as in the management of patients.

Chapter 2

E-CADHERIN AND GASTRIC CANCER

2.1 E-cadherin and Metastasis

Cadherin is a superfamily of calcium-mediated membrane glycoproteins, forming one of the four classes of adhesion molecules. Some common cadherins expressed by epithelial cells are E-cadherin, N-cadherin, and P-cadherin. The cadherin binds to cytosolic proteins namely α- catenin, β- catenin, and, γ-catenin which in turn links to the actins to form the intracytoskeleton (Takeichi et al, 1990; Grunwald et al, 1993). The cadherins are responsible for the homotypic cell-cell adhesion. In carcinogenesis, the tumour cell has to dissociate from one another before it can invade or metastasize. Therefore, these adhesion molecules are thought to play an important role in carcinogenesis and metastasis. E-cadherin is expressed in all epithelial cell types. Under-expression of the E-cadherin is found in gastric, hepatocellular, oesophageal, breast, prostatic, bladder and gynaecological carcinomas and correlates with infiltrative and metastatic ability (Takeichi et al, 1993). It has been postulated that the under-expression of E-cadherin in these tumours may account for their invasive potential and appear to be a late event. Hence, the E-cadherin gene is also called an invasion suppressor gene.

2.2 Cadherin-catenin Complex and Oncogenesis

The observation that certain human cancers expressing an abundance of cadherins can metastasize poses the question of how they leave the primary tumour. One possible mechanism for such a process would be a transient and local loss of cadherins due to down regulation of the expression or proteolysis. Another possible mechanism is pertubation of the cadherin cell adhesion system without loss of cadherin. α-Catenins is

lacking in the lung carcinoma cell line PC9, despite the expression of E-cadherin, these cells cannot tightly associate. Pertubation of the cadherin adhesion system may also occur as a result of biochemical modification of catenins. Phosphorylation of catenins might interfere with cadherin action bringing about unstable cell-cell adhesion. It has been shown that epidermal growth factor receptor, c-erb-2, hepatocyte growth receptor c-met, and the oncoprotein $pp60^{vsrc}$ can phosphorylate β-catenin.

2.3 Expression of E-cadherin in Gastric Cancer

The expression of E-cadherin has been studied by immunohistochemical method. Decreased expression has been observed in gastric cancer by various authors, ranging from 17% (Shimoyama and Hirohashi, 1991) to 92% (Mayer et al., 1993), depending on the method and the definition used. Direct correlation between E-cadherin and the grade of tumour differentiation has also been observed in all these studies. In addition, it was shown in a study of 413 gastric cancers by Gabbert et al (Gabbert et al., 1996) that patients with E-cadherin positive tumours had significantly better 3- and 5-year survival rates than patients with E-cadherin negative tumours. The decreased expression of E-cadherin was mainly observed in diffuse type and less in intestinal type of gastric cancer. One possible mechanism for the decreased expression is mutation in the E-cadherin gene and/or loss of heterozygosity in 16q22.1 (Becker et al, 1993; Becker et al, 1994; Becker et al, 1995; Muta et al, 1996). Mutations of the E-cadherin gene have been reported in 50% of diffuse carcinomas of the stomach (Becker et al, 1994). Recently, the report of germline mutations in kindred with early onset diffuse gastric carcinoma became the first description of a molecular basis for familial gastric cancer of the diffuse type (Guilford et

al, 1998). The other possible mechanism of decreased cadherin expression is by CpG methylation of the cadherin gene (Tamura et al, 2000).

Chapter 3

SOLUBLE E-CADHERIN IN GASTRIC CANCER

3.1 Introduction

Gastric cancer remains the second major cause of cancer-related deaths in the world. However, there is currently no satisfactory tumour marker for diagnosis or monitoring the disease progress. The most frequently used tumour markers in gastric cancer are carcinoembryonic antigen (CEA) and CA19-9, but only a modest proportion of patients has elevated levels of these markers.

The cadherins are a major class of adhesion molecules which play an important role in the homotypic cell-cell adhesion and hence cancer cell metastasis and invasion. E-cadherin is a member of the cadherin family which is expressed in all epithelial cells. The role of E-cadherin in metastasis and invasion could be evidenced by the fact that the invasiveness of epithelial tumour cell lines could be inhibited *in vitro* by transfection and expression of E-cadherin cDNA, and induced again by exposure to anti-E-cadherin monoclonal antibodies (Behrens et al, 1989; Frixen et al, 1991; Vleminckx et al, 1991). Under-expression of the E-cadherin molecule has been found in various malignancies and has the potential value to be a prognostic marker (Takeichi, 1993).

Serum soluble E-cadherin is the degradation product of the cellular E-cadherin molecule. It is found in the circulation of normal individuals but is particularly elevated in patients with malignancies. Serum soluble E-cadherin has been shown to be a potentially valuable prognostic marker for carcinoma of bladder (Griffiths et al, 1996). However, its prognostic value has not been proven in colorectal cancer (Velikova et al, 1998), and its value in gastric cancer has been controversial. Velikova et al (1997) did not show

significant difference between serum soluble E-cadherin in patients with gastric cancer and normal subjects, while Gofuku et al (1998) showed that the concentration was significantly elevated in 67% of patients.

3.2 Objectives

This chapter is going to report the work for the following objectives:

1. To confirm the observation that serum soluble E-cadherin is present in higher concentration in patients with gastric cancer than in normal subjects.
2. To define the range of soluble E-cadherin level in both Chinese patients with gastric cancer and normal subjects.
3. To investigate the value of serum soluble E-cadherin as a prognostic marker in patients with gastric cancer.

3.3 Materials and Methods

3.3.1 Patient selection

All patients admitted from 1st January 1997 to 30th September 1998 into the Departments of Medicine and Surgery, Queen Mary Hospital with histologically proven gastric carcinoma, including both operable and inoperable tumours, were recruited. The sera of 125 patients were collected after gastric cancer was confirmed histologically and before operation or initiation of chemotherapy. Nine patients were excluded from the present analysis because two of them were non-Chinese, one had serum collection after tumour debulking, one had another synchronous tumour and five had coincidental liver cirrhosis.

Therefore, the total number of patients included in the analysis was 116. A group of 40 healthy subjects were recruited as control.

3.3.2 Patient Assessment

After gastric cancer was confirmed histologically by endoscopic biopsy, the extent of disease was assessed by chest X-ray, endoscopic ultrasound and computer tomography or ultrasound of the abdomen.

3.3.3 Definitions of Treatments

Curative resection was defined as UICC R0 resection. Palliative treatment included UICC R1 or R2 resection, gastrojejunostomy or palliative chemotherapy. Conservative treatment referred to patients receiving symptomatic support only.

3.3.4 Staging and Classification of Gastric Cancer

Tumour was staged according to the Japanese Research Society for Gastric Cancer criteria (Kanehara 1995) and classified histologically according to the World Health Organisation and the Lauren's Classification (1965).

3.3.5 Assay of Soluble E-cadherin

Venous blood samples were collected into plain tubes, allowed to clot and within 1 hour of collection were centrifuged at 800g for 10 min at 4 ^{o}C to obtain the serum. The serum was removed, aliquoted and stored at –70 ^{o}C until assay. The concentration of soluble E-cadherin was measured with a commercially available sandwich ELISA kit based on monoclonal antibodies (Zymed® Laboratories Inc., South San Francisco, USA). All blood

samples were measured by an investigator who was blinded from the clinical details and the coded-data sheet. Each sample was measured twice.

The method of assay was described elsewhere (Katayama et al, 1994), but briefly as follows: The first monoclonal antibody, HECD-1 was coated onto the microtitre-plate wells to create the solid phase. Non-specific binding was blocked by a blocking buffer. Serum samples from patients and the standard solutions supplied were incubated in the microtitre-plate wells. The second monoclonal antibody SHE 13-1 labelled with peroxidase was added. During incubation, human E-cadherin molecule was trapped by the two monoclonal antibodies as a sandwich. The reaction between the peroxidase and the substrate solution (H_2O_2 and tetramethybenzidine) resulted in colour development with intensities proportional to the concentration of human E-cadherin present in the samples and standards. The colour developed was measured with the microtitre-plate reader for measurement of absorbance at 450nm. Accurate sample concentrations of human E-cadherin were determined by comparing the specific absorbances with those obtained from the standards plotted on a standard curve.

3.3.6 Statistical Methods

Data were collected and analysed by Statistical Package for Social Sciences. Logarithmic transformation was performed on the data of soluble E-cadherin to convert into normal distribution. Clinical and biochemical parameters of patients were expressed as means ± SD. Comparisons were performed with the independent sample Student's t test and Chi-square test. Differences were considered significant when $p < 0.05$, and approaching

statistical significance when $p < 0.1$ and ≥ 0.05. Cut-off values of soluble E-cadherin concentration were calculated by discriminant analysis.

3. 4 Results

There were 75 men and 41 women in the patient group with a mean age of 66 ± 14 years. Nineteen men and 21 women were in the healthy control with a mean age of 31 ± 10 years. The size of tumours measured from the pathological specimens obtained after resection ranged from 0.5 cm to 18 cm (mean = 4.8 ± 3.2 cm). Forty-eight percent of tumours were located in the gastric antrum. Of those with gastric resection specimen available for pathological examination, 61% were intestinal type, 30% were diffuse type and 9% were mixed type according to Lauren's classification. The percentage of patients at stage I, II, III and IV diseases were 12.6%, 18.4%, 28.2% and 40.8%, respectively. Therefore, most of our patients presented at the advanced stages.

The means of the logarithm of soluble E-cadherin concentration in patients with gastric cancer were significantly higher than normal healthy subjects (3.85 ± 0.28 vs 3.71 ± 0.18, $p = 0.001$). On the other hand, the means of the logarithm of soluble E-cadherin concentration in patients with T4 invasion, liver metastasis, distant metastasis, and stage III/IV disease were higher than the means of other tumour depth invasion, absence of liver metastasis, absence of distant metastasis, and stage I/II disease, respectively, with p value approaching to statistical significance ($p = 0.057$, 0.067, 0.082, 0.086, respectively). Logarithm of soluble E-cadherin concentration correlated with the size of

tumours ($p = 0.032$). It also correlated with the logarithm of carcinoembryonic antigen (CEA) concentration ($p = 0.001$).

Fifty-four patients underwent curative gastric resection while 43 patients received palliative treatment. Another ten patients received conservative treatment only. Nine patients were excluded from further analysis of their treatment results because eight of them were operable but medically unfit and one committed suicide before receiving any treatment. The means of the logarithm of soluble E-cadherin concentrations in patients receiving palliative/conservative treatment and those receiving curative resection were 3.91 ± 0.35 and 3.78 ± 0.19, respectively ($p = 0.015$).

The cut-off value of serum soluble E-cadherin of normal subjects and patients with gastric cancer was calculated to be 5994 ng/ml. Twenty-seven and a half percent of the normal subjects and 51.7% of the patients were above this cut-off value. The cut-off value for curative treatment and palliative/conservative treatment was calculated to be 7025 ng/ml. Concentration higher than 7025 ng/ml was used to predict the relative risks of various poor prognostic factors (Table 1). Patients with soluble E-cadherin concentration above the cut-off value were more likely to have T4 invasion ($p = 0.020$, C.I. = 1.008-1.668) and palliative/conservative treatment ($p = 0.023$, C.I. = 1.038-2.514). The relative risks of N2 metastasis, distant metastasis and stage III/IV disease were 1.41, 1.33 and 1.55, respectively ($p = NS$).

3.5 Discussion

During carcinogenesis, tumour cells have to dissociate from one another before they can invade or metastasise. Therefore, adhesion molecules are expected to play an important role in carcinogenesis and especially in metastasis. Decreased membranous expression of E-cadherin molecules has been found in gastric cancer (Shimoyama and Hirohashi, 1991; Mayer et al, 1993) and other malignancies like colon (Dorudi et al, 1993; Gagliardi et al, 1995) pancreas (Pignatelli et al, 1994), oesophagus (Jankowski et al, 1994), liver (Kozyraki et al, 1996), prostate (Umbas et al, 1994), bladder (Bringuier et al, 1993; Syrigos et al, 1995), breast (Moll et al, 1993; Siitonen et al, 1996), and head and neck tumours (Andrews et al, 1997). The disruption of the membranous expression of E-cadherin could be caused by disturbed polarization of the cell or due to mutations or partial deletions of the E-cadherin gene (Becker et al, 1993), resulting in a protein which is not transported to the cell membrane. Direct correlation between E-cadherin and the grade of tumour differentiation has been observed in some of these tumours (Umbas et al, 1994; Bringuier et al, 1993; Syrigos et al, 1995). In gastric cancer, it was shown in a multivariate retrospective study of 413 patients that E-cadherin positive tumours had significantly better 3- and 5-year survival rates than E-cadherin negative tumours (Gabbert et al, 1996).

Soluble E-cadherin, a 80 kDa peptide, is considered to be the degradation product of the 120 kDa intact E-cadherin generated by a Ca^{2+} ion-dependent proteolytic action (Wheelock et al, 1987; Takeichi et al, 1988). The peptide was found in the circulation of healthy persons and was not dependent on age or sex. It was elevated in patients with

gastric carcinoma and other malignancies (Katayama et al, 1994). Increased serum soluble E-cadherin concentration has also been found in pemphigoid or pemphigus skin condition (Furukawa et al, 1997; Shirahama et al, 1996; Matsuyoshi et al, 1995) and in multi-organ failure (Pittard et al, 1996). Since E-cadherin is expressed in all epithelial cells, any condition with rapid epithelial cell turn-over may lead to an increase in its concentration. Therefore, patients in these conditions and with chronic inflammation diseases were excluded from our study. In addition, patients with cirrhosis were also excluded from the present study because we believe cirrhosis could be another condition in which cells turn over rapidly and may result in higher levels of soluble E-cadherin. This has been confirmed in our unpublished data. However, it is also important to identify other conditions that may significantly affect soluble E-cadherin concentration.

The present study confirmed the observation that the concentration of soluble E-cadherin in patients with gastric cancer was higher than that in healthy subjects. However, higher soluble E-cadherin concentrations were observed in both our healthy subjects and patients than those reported in the literature, with mean value of 5616 ng/ml *vs* 2515 ng/ml in healthy subjects, and 9344 ng/ml *vs* 4735 ng/ml in patients with gastric carcinoma (Gofuku et al, 1998). Only Chinese subjects were recruited in the study because we do not know whether racial difference will have any effect on the soluble E-cadherin concentration. Difference in biological behaviour in gastric cancer between Japan and the Western world has been suggested and might account for the differences observed in prognosis (Livingstone et al, 1995). Therefore, the higher E-cadherin concentration in our patients and normal controls could be due to racial differences. In

addition, the fact that most of our patients have advanced diseases may also partly explain the high concentration of soluble E-cadherin in them. Consequently, each laboratory should have its own reference range.

Our results showed that soluble E-cadherin concentrations were elevated in patients receiving palliative/conservative treatment, and were correlated with the size of gastric tumour. Patients with soluble E-cadherin concentration higher than 7025 ng/ml cut-off value were more likely to have non-curative resection possibly due to T4 invasion. In addition, these patients were more likely to have stage III and IV disease, although statistical significance was not reached. However, this observation was biased by the fact that a large number of patients with advanced diseases were inoperable and therefore their diseases could not be staged, thus rendering the results less significant. Tumour size, depth of tumour invasion, and operability are important prognostic factors in patients with gastric carcinoma. Tumour size has been reported as a simple prognostic indicator for gastric carcinoma (Adachi et al, 1997). Soluble E-cadherin might originate from the rapid turn-over of the tumour cells. Therefore, the bigger the tumour size is, the higher the soluble E-cadherin concentration would be. The increase in relative risks of metastases in patients with higher soluble E-cadherin concentration reflected the role of E-cadherin as an ‘invasion suppressor molecule’ (Takeichi et al, 1993; Birchmeier et al, 1994).

Our data showed that serum soluble E-cadherin correlated with poor prognostic markers. High serum soluble E-cadherin predicted T4 invasion and palliative/conservative

treatment. However, it was only elevated in a subgroup of patients. Therefore, further studies should aim at identifying the subgroup of gastric cancer patients who have elevated soluble E-cadherin concentration, thereby increasing its sensitivity; comparing the prognostic value of soluble E-cadherin and conventional markers such as carcinoembryonic antigen in patients with gastric cancer; and also identifying other conditions that may affect the soluble E-cadherin concentrations. In addition, prospective studies should also be carried out to investigate the post-treatment soluble E-cadherin level and its role in therapeutic monitoring in patients with gastric cancer.

Table 1: Prediction of relative risks of various prognostic factors using concentration of soluble E-cadherin higher than the cut-off value (> 7025 ng/ml).

Factors	**Categories**	**Relative risk**	**P value**	**Confidence interval**
T	T4 *vs* T1 or T2 or T3	1.30	**0.020**	**1.008-1.668**
N	N2 *vs* N0 or N1	1.41	0.064*	0.944-2.118
M	M1 *vs* M0	1.33	0.073*	0.954-1.859
Staging	Stage III or IV *vs* Stage I or II	1.55	0.164	0.82-2.913
Operability	Palliative/conservative *vs* Operable	1.62	**0.023**	**1.038-2.514**

T = tumour N = lymph node M = metastasis

P < 0.05 is considered statistically significant and was indicated by bold type character. P < 0.1 but > 0.05 is considered approaching statistical significance and was marked with an asterisk.

Chapter 4

CONCLUDING REMARKS

The understanding of molecular biology and molecular genetics leads to a fundamental change in the practice of medicine. The underlying pathology and behaviour of the tumour are better understood with molecular study than by its clinical appearance alone. The present pathological classification systems for gastric cancer and other tumours depend mainly on the morphology and histology. They are not completely satisfactory because they cannot accurately predict and reflect the biological and clinical behaviour of the tumour. The molecular markers may be used to identify disease subgroups with differing natural history or response to various treatments.

The use of molecular markers as tumour markers and prognostic markers seems to be most promising. The study of soluble E-cadherin reported herein demonstrated the potential role as a prognostic marker in identifying patients with advanced gastric cancer. However, it should be noted that, in the study, soluble E-cadherin was shown to correlate with known poor prognostic markers, and not identified as an independent marker. Further studies are necessary for investigating its correlation with survival.

Nevertheless, a number of other molecular markers have already been used extensively in clinical practice. Carcinoembryonic antigen (CEA) is a 180kDa adhesion molecule, CD66e, which belongs to the immunoglobulin superfamily. It is one of the most extensively studied markers. Increased CEA has been shown to be characteristic of metastatic colonic carcinoma (Jessup et al, 1989). Whereas carbohydrate antigen 19-9 (CA 19-9) has also been reported to be significantly related to metastatic colonic carcinoma (Nakayama et al, 1995, Shimono et al, 1994). This is a product of the Lewis

gene and serves as a ligand for members of the selectin family. There are other newly discovered adhesion molecules like CA125 and CA72-4, which are already being used in daily clinical practice. Other potential markers are emerging such as PAI-1, MMP-2, c-erbB-2, p53.

However, the cost for molecular study at present is expensive. The issue on cost-effectiveness has not been properly assessed. Nevertheless, molecular biology is becoming the major field for development in the near future and the fundamental practice of medicine would have to be changed.

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