Expression of Autocrine Motility Factor Receptor and E-Cadherin in Gastric Adenocarcinoma-A Correlative study

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Abstract: Carcinoma of the stomach is still remained among the most common cause of cancer death worldwide. In an effort to understand interaction between force of Intercellular adhesion and its disruption by propagating cancer cells we have selected 46 caresses of Gastric adenocarcinomas. As E-cadherin (ECD) is the strongest intercellular adhesion molecule in nonmalignant Gastric mucosal epithelial cell and Autocrine Motility Factor (AMF) is an established propagator of its malignant counterpart, we have studied simultaneous expression of ECD and AMFR (Autocrine motility Factor Receptor). With aid of immunohistochemical technique, strong or weak expression of ECD and AMFR was determined according to predetermined parameters. Normal Gastric mucosal cell (the control cases) strongly express ECD whereas reverse was true with AFMR. We find decrease ECD and simultaneous increase of AMFR in gastric adenocarcinomas. Furthermore, more aggressive morphologic variant showed varied expression in comparison to its less aggressive type. Diffuse type of Gastric adenocarcinoma show weak ECD (61.5% vs 40%) and more AFMR (53.8% vs 30%) expression in comparison to Intestinal type. Strong AMFR expression is correlated positively with increasing depth of invasive tumor. As both ECD and AMFR are involved in the pathway of tumor progression as well as development of more aggressive type of Gastric Adenocarcinomas, their simultaneous examination is necessary to evaluate biologic potential of this tumor.

Key words: Gastric Adenocarcinomas; E-Cadherin; Autocrine motility Factor Receptor

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I. Introduction

Adenocarcinoma of the stomach, a leading cause of cancer death worldwide is the second and fourth most common cancer in males and females respectively (1,2). In Asia, such incidence in male is similar, whereas it ranked third among female (3). Globally, gastric cancer accounts for about 1 lakh new cases and close to 0.8 lakh deaths annually. The case-fatality ratio of gastric cancer is higher than for common malignancies like colon, breast, and prostate cancers (4). Despite advances in diagnosis, the disease is usually detected after invasion of the muscularis propria, because most patients experience vague and nonspecific symptoms in the early stages and the classic triad of anemia, weight loss, and refusal of meat-based foods is seen only in advanced stages. Furthermore, surgery and chemotherapy have limited value in advanced disease and there is a paucity of molecular markers for targeted therapy. Since cancer of the stomach has a very poor prognosis and the 5-year survival rate is only around 20 per cent, a new look at the results of epidemiological and experimental studies is important to establish strategies for early precise detection and prognosis (5).

Obviously, despite such prevalence of malignant lesions, modern science yet to find complete curative treatment protocol of neoplasm. Limited success of medical science in such curative treatment is restricted to mostly early staged and low graded lesions. At cellular level, progression of malignancy is dependent variably on many cellular properties including intercellular adhesion, motility and proteolysis (6,7) for infiltration of malignant cell into surrounding stroma, reduction of intercellular adhesion and increment of cell motility appeared two necessary simultaneous incidents.

It is established that E-cadherin (ECD) is strongest intercellular adhesion molecule in epithelial cell (6,8) and such adhesion is regulated by ECD and ECD associated proteins including catenin (18,19). Many researchers have indicated correlation between infiltration of malignant cell and diminished ECD and catenins both in vitro and in vivo in malignant lesion of various organs (18,19) including esophagus, stomach, breast and colon (20-23).

As previous researchers already confirmed that Malignant cell infiltration is modulated by property of cell motility-which in turns is affected by various motility factors like Hepatocyte Growth factor, Epidermal growth factors (24-26) and as Silleti et al found that loss of intercellular adhesion up regulate the proteine...
expression and promoter activity of AMFR(35), we intend to find efficacy of AMFR in malignant cell progression.

**Autocrine Motility Factor (AMF)** has been purified from the culture media of various tumor cells as a specific motility modifier.(27,28) The receptor for AMF (AMFR) has been identified as a cell surface glycoprotein (gp78; molecular weight, 78,000) on the B16-F1 melanoma cell line with high metastatic ability.(27,28) Autocrine Motility Factor Receptor (AMFR) concentrates on the leading edge of the cell surface, then is phosphorylated and internalized by binding with AMF.(29) Finally, it induces rearrangement of integrin, causing cells to move.(30) In this pathway, G protein might be involved, since cell motility is inhibited by a Bordetella pertussis toxin.(30) Up-regulation of AMFR and its implication in cancer progression in human cancers of various origin, including the large intestine,(31) placenta,(32) esophagus,(33) and stomach,(31,34) has been reported.

We intend to study simultaneous destruction of cellular adhesion and effect of motility factors in propagation malignant cell of epithelial origin. Review of literature revealed in epithelial cell, ECD is strongest intercellular adhesion molecule(36), association between ECD and AMFR is studied in various epithelial malignancies i.e. carcinomas and simultaneous loss of ECD and increase in AMFR is found in cultured cell lines of Urinary Bladder carcinomas.(37) This simultaneous alteration of ECD and AMFR, if they are situated on the common signal, enables us to understand that cancer progression more fluently leads to invasion and metastasis.

It is established that Gastric carcinomas depending on their diverse morphology, cellular origin and prognosis are divided into two categories—namely Intestinal and Diffuse type.

In our study, we have evaluated expression of ECD and AMFR gene with aids of Immunohistochemistry. We found that the difference in natural history of these two type of gastric carcinomas can be partially explained by behavioral pattern of ECD and AMFR.

### II. Materials And Methods

The study population consisted of 46 patients who were finally treated with total or distal gastrectomy with or without regional lymph node dissection. In this retrospective study (conducted between 2010 to 2015), in Medical College, Kolkata, we selected only those patients who underwent endoscopic evaluation followed by final surgery. Interval between endoscopy and final surgery in our study varied from 22 to 166 days. Most patients underwent final surgery within 50 days of the endoscopic evaluation. To reduce influence on natural history the disease, we selected only patients who have received no anticancer therapy prior to the surgery.

Stomach cancer incidence is known to increase with age with the peak incidence occurring at 60-80 years. Cases in patients younger than 30 years are very rare (6,7). In India, the age range for stomach cancer is 35-55 years in the South and 45-55 years in the North. The disease shows a male preponderance in almost all countries, with rates two to four times higher among males than females(8). In the present study, Age of the patients ranged between 36 to 74 years (male n=35 and female n=11), roughly in accordance to the previous results.

Clinical data including copy of histopathology requisition slips were collected from tertiary treatment center in Kolkata. Fresh Copy of Hematoxyline and Eosin stained tissue sections of endoscopic biopsy and total or distal gastrectomy with or without regional lymph node dissection specimens were prepared from paraffin blocks. Team of physicians, surgeons and Pathologist in Medical College, Kolkata went through the clinical data and tissue sections as per previously fixed protocol and parameters.

Cases in which histologic slides from endoscopic biopsy were not available for review were excluded. Pathologic Staging and Grading was performed by the team pathologist according to the WHO classification system TNM (tumor, lymph nodes, and metastasis) system.

Such representative Hematoxyline and Eosin stained tissue 0.5 micrometer thick sections were studied and Tumour was designated intestinal or diffuse type based on histomorphology. Depth of Tumour invasion was noted following established WHO Guideline. Sections for immunohistochemistry was selected among the paraffin blocks which were taken from invasive margins of tumor, and had tumor in 50% or more of total section area.

Immunohistochemistry was performed on sections obtained from representative block of formalin-fixed paraffin-embedded tissue using the Avidin-biotin complex technique. The sections were deparaffinized in xylene, and rehydrated in a graded ethanol series. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. The slides were subsequently incubated at room temperature with reagents. After washing in a 0.05-mol/L concentration of phosphate-buffered saline (PBS), they were incubated with 3% normal rabbit serum for AMFR or 3% normal mouse serum for ECD for 30 minutes to block nonspecific conjugation in the tissues. The specimens were incubated sequentially with the primary anti-AMFR monoclonal antibody, 3F3A19, or antihuman ECD antibody, HECD1 (Takara Shuzo, Kyoto, Japan), at 4°C overnight. After washing with PBS, they were incubated with biotinylated rabbit antiratIgG for AMFR or rabbit antimouseIgG (Vectastain ABC Kit, Vector, Burlingame, CA), diluted 1:250 in PBS, for 30 minutes at room temperature and with ABC reagent.
Expression of Autocrine Motility Factor Inhibitor Receptor (AMFR) and E-Cadherin in Gastric Adenocarcinoma - A.

During immunohistochemical evaluation of ECD and AFMR tumour cell were designated positive or negative as per predetermined criteria (Table 1). For statistical analysis, differences between the 2 groups were assessed by the Mann-Whitney U test, and correlations between 2 parameters were evaluated by the Spearman rank correlation test.

III. Results

In non malignant gastric mucosa, ECD is strongly expressed at intercellular border. In contrast to ECD expression, AMFR, in such cases is seen in some foci of proliferating zone. (Image 1, 2, 3)

In gastric cancer cells, AMFR frequently was expressed in the cell surface and cytoplasm, (image 4, 5) and ECD expression frequently was reduced in a homogenous or heterogeneous fashion (Image 6, 7). Thus, the alteration in gastric cancers was follows: 20 cases (43.4%) showed strong expression of AMFR, and 24 cases (52.1%) showed weak ECD expression.

The expressions of AMFR and ECD molecules were correlated with morphologic variant as well as depth of tumor invasion in Gastric Adenocarcinoma (Table 2). Strong expression of AMFR was observed more frequently in diffuse-type carcinomas (14/26 [53.8%]) than in intestinal- type carcinomas (6/20 [30%]). Likewise, the frequency of weak expression of ECD was higher in diffuse type carcinomas (16/26 [61.5%]) than in intestinal-type carcinomas (8/20 [40%]). The alterations of these molecules were associated with diffuse-type carcinomas, which imply a loss of differentiation (P = .005 and P = .0223 for AMFR and ECD respectively). Strong expression of AMFR was observed less frequently in superficial (T1) cancer (5/18 [27.7%]) than those with deeper infiltration (T2,3) (14/26 [53.8%]). There was a significant positive correlation between the depth of invasion and the expression of AMFR (P = .0382); however, the proportion of ECD reduction (weak expression) was similar in superficial and deep infiltrating tumors.

When the expression of ECD and AMFR are compared (Table 3), strong expression of AMFR was more frequent in tumors with weak expression of ECD (14/24 [58.3%]) than in tumors with strong expression of ECD (6/22 [27.2%]), thereby showing a significant negative correlation (P = .0033). When other morphometric parameters were reevaluated according to the coexpression pattern of these molecules, tumors with strong AMFR and weak ECD expression showed deep tumor invasion (T2,3) more frequently than tumors with weak AMFR and strong ECD expression.

**Table 1**

Evaluation of Autocrine Motility Factor Inhibitor Receptor (AMFR) and E-Cadherin (ECD) Expression

<table>
<thead>
<tr>
<th>Autocrine Motility Factor Inhibitor Receptor (AMFR)</th>
<th>Strong Expression</th>
<th>Weak Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Cadherin (ECD)</td>
<td>50% or more tumor cell stained</td>
<td>Less than 50% of tumor cell stained</td>
</tr>
<tr>
<td>90% or more tumor cell stained</td>
<td>Less than 90% of tumor cell stained</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

Expression of Autocrine Motility Factor Inhibitor Receptor (AMFR) and E-Cadherin (ECD)

<table>
<thead>
<tr>
<th>Histopathologic Type</th>
<th>AMFR Strong</th>
<th>AMFR Weak</th>
<th>ECD Strong</th>
<th>ECD Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal (n=20)</td>
<td>6</td>
<td>14</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Diffuse (n=26)</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth of Invasion</th>
<th>AMFR Strong</th>
<th>AMFR Weak</th>
<th>ECD Strong</th>
<th>ECD Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (n=18)</td>
<td>5</td>
<td>13</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>T2 (n=17)</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>T3 (n=9)</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table 3**

Relationship Between Autocrine Motility Factor Inhibitor Receptor (AMFR) and E-Cadherin (ECD) Expression

<table>
<thead>
<tr>
<th>AMFR</th>
<th>ECD Strong</th>
<th>ECD Weak</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMFR Strong</td>
<td>6</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>AMFR Weak</td>
<td>16</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>24</td>
<td>46</td>
</tr>
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IV. Discussion

Histologically, gastric cancers are classified into intestinal type and diffuse type. The former arises from intestinal metaplasia of foveolar epithelium and from the tubules, and the latter arises from the proper gastric gland and shows a diffuse growth pattern.(38)

The E-cadherins (ECD), or “classical” cadherins of type I, belong to the large family of cadherins, transmembrane or membrane-associated glycoproteins, mediating cell-cell adhesion and playing a pivotal role in epithelial cell behavior and tissue morphogenesis or remodeling (9–15). Transcriptional ECD reprogramming in epithelial cells leads to decreased adhesion and enhanced migration or invasion at the epithelial-to-mesenchymal transition (EMT) interface during cancer progression (16).

As for ECD, it is the characteristic of diffuse type tumors that the function of ECD is disturbed, even in the presence of its protein expression,(21) because of ECD gene mutation or tyrosine phosphorylation of ECD binding proteins.(19) Accordingly, as mentioned previously, loss of cell-cell adhesion induces transcription of the AMFR gene. In the present study, we found more AMFR overexpression in diffuse-type tumors than in intestinal-type tumors. This probably is a consequence of a functional or expression disorder of ECD.

As ECD is normally expressed on cell surface, it was advantageous to set the cutoff line at 90% for ECD expression.(31,39) However, as AMFR was expressed only slightly in normal epithelium and gradually increased in cancer cells, a 50% cutoff was sufficient for separating AMFR expression into 2 groups.(19)

In the present study, we found overexpression of AMFR in about half of the patients with gastric cancer and association of AMFR with dedifferentiation and deep tumor infiltration. In 1 study that examined the role of AMFR in gastric cancers,(34) the observations were consistent with ours.

The mechanism for regulation of AMFR is yet to be known in detail. The AMFR gene is located on 16q2130. In cultured cell lines, cell-cell contact dramatically down-regulated the protein expression and messenger RNA transcription of the AMFR gene.(35) Researchers performed an AMFR promoter assay and found it was suppressed by high cell density. They could not identify the transcription factor but speculated that c-Myc was a candidate, since the amount of c-Myc was correlated inversely with cell density.(40) There is another report that retinoic acid down-regulates AMFR expression.(41) Since retinoic acid induces differentiation in various types of cells, differentiation might be another factor that regulates AMFR expression.

These phenomena convinced us that ECD is involved in transcriptional regulation of AMFR. For example, ECD is the strongest cell-cell adhesion molecule(36) and beta-catenin, an ECD binding protein, is reported to be associated with c-Myc transcription.(42) Retinoic acid is known to up-regulate ECD expression.(43) Although the suppression of AMFR transcription by ECD has not been proven directly, the inverse correlation of ECD and AMFR expression has been reported in bladder carcinomas.(44) and we found the same relationship in human gastric cancers. Since ECD itself is a strong repressor of cancer invasion and metastasis, the reduction of ECD induces cancer invasion and metastasis, both by the function itself and by the regulatory mechanism for AMFR expression.

In conclusion, complicated histologic types in gastric cancers and their properties could be understood partly by the expression of ECD and AMFR in the present study. We find importance of evaluation of both molecules simultaneously, and the synergistic effect of these molecules seems to be a crucial step for progression of malignancy of epithelial origin.

Images

Figure 1: (Magnification = x400) Normal AMFR expression in gastric mucosa
Expression of Autocrine Motility Factor Receptor and E-Cadherin in Gastric Adenocarcinoma-A....

Figure 2: (Magnification = x400) Normal ECD expression in gastric mucosa

Figure 3: (Magnification = x400) Normal ECD expression in gastric mucosa with Intestinal Metaplasia

Image 4: (Magnification = x400) AMFR expression in Diffuse type of Gastric Carcinoma
Expression of Autocrine Motility Factor Receptor and E-Cadherin in Gastric Adenocarcinoma-A.….  

Image 5: (Magnification = x400) AMFR expression in Intestinal type of Gastric Carcinoma

Image 6: (Magnification = x400) ECD expression in Intestinal type of Gastric Carcinoma

Image 7: (Magnification = x400) ECD expression in Diffuse type of Gastric Carcinoma

Reference


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