ANALISI CLINICO-PATOLOGICA E MOLECOLARE
DELL’ADENOCARCINOMA DELLA GIUNZIONE
ESOFAGO-GASTRICA IN PAZIENTI CON ETÀ INFERIORE A 40 ANNI

CLINICOPATHOLOGICAL AND MOLECULAR CHARACTERIZATION
OF GASTROESOPHAGEAL JUNCTION (GEJ) ADENOCARCINOMA
BEFORE AGE OF 40 YEARS

Tesi di Dottorato di: ALBERTO RUFFATO
Relatore: Chiar.mo Prof. SANDRO MATTIOLI

Supervisori:
Dr. VALERIE W. RUSCH
Dr. LAURA H. TANG

Coordinatore: Chiar.mo Prof. SANDRO MATTIOLI

MED/21 CHIRURGIA TORACICA

Anno Accademico 2005-2006
SUMMARY

Gastroesophageal junction (GEJ) adenocarcinoma are uncommon before age of 40 years. While certain clinical, pathological, and molecular features of GEJ adenocarcinoma in older patients have been extensively studied, these characteristics in the younger population remain to be determined. In the recent literature, a high sensitivity and specificity for the detection of dysplasia and esophageal adenocarcinoma was demonstrated by using multicolor fluorescence in situ hybridization (FISH) DNA probe set specific for the locus specific regions 9p21 (p16), 20q13.2 and Y chromosome.

We evaluated 663 patients with GEJ adenocarcinoma and further divided them into 2 age-groups of ≤ 40 and ≥ 50 years, respectively. FISH with selected DNA probe for Y chromosome, locus 9p21 (p16), and locus 20q13.2 was investigated with formalin fixed and paraffin embedded tissue from surgical resections of 17 younger and 11 older patients. Signals were counted in > 100 cells with each given histopathological category. The chromosomal aberrations were then compared in the 2 age-groups with the focus on uninvolved squamous and columnar epithelium, intestinal metaplasia (Barrett’s mucosa), glandular dysplasia, and adenocarcinoma. Comparisons were performed by the χ2 test, Fisher's exact test, Student's t-test and Mann-
Whitney U-test as appropriate. Survival was estimated by the Kaplan-Meier method with univariate analysis by the log-rank. Significance was taken at the 5% level.

There was no difference in the surgical technique applied in both age groups and most patients underwent Ivor Lewis esophagectomy. Among clinical variables there was a higher incidence of smoking history in older patient group. We identified a progressive loss of Y chromosome from benign squamous epithelium to Barrett’s mucosa and glandular dysplasia, and, ultimately, to a near complete loss in adenocarcinoma in both age groups. The young group revealed significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older patients group. In addition, we demonstrated an increase in the percentage of cells showing gain of locus 20q13.2 with progression from benign epithelium through dysplasia to adenocarcinoma with almost the same trend in both the young and the older patients.

When compared with the older age-group, younger patients with GEJ adenocarcinoma possess similar known demographics, environmental factors, clinical, and pathologic characteristics. The most commonly detected genetic aberrations of progressive Y chromosomal loss, 9p21 locus loss, and 20q13 gains were similar in the younger and older patients. However the rate of loss of 9p21 is significantly higher in
young patients, in both the benign and the neoplastic cells. The loss of 9p21, and possibly, the subsequent inactivation of p16 gene may be one of the molecular mechanisms responsible for the accelerated neoplastic process in young patients.
INTRODUCTION

A rapid increase in the incidence of adenocarcinoma of the distal esophagus and gastroesophageal junction (GEJ) has been observed in Western countries in the last fifteen years. This has reanimated the debate on possible etiologic factors, early diagnosis, and treatment for these tumors (1-4). A number of hypotheses have been proposed with regard to risk factors for the development of GEJ adenocarcinomas that broadly can be divided as environmental and genetic influences (5, 6). It is well recognized that patients with longstanding reflux esophagitis and subsequent intestinal metaplasia (Barrett’s esophagus) are at risk for the development of adenocarcinoma that arises within the context of progressive molecular alterations extending through low and high grade dysplastic stages (7-9). However, the incidence of adenocarcinoma in Barrett’s esophagus is <5% per year (10) thus clearly Barrett’s mucosa cannot be the only criterion to recognize patients who are at risk for acquiring esophageal adenocarcinoma. Therefore, prognostic parameters which can reliably predict malignant progression in Barrett’s esophagus are required. It is well documented that GEJ adenocarcinoma is most commonly diagnosed in white male patients in their sixth and seventh decades of life. The median age for patients with adenocarcinoma treated by esophagectomy is 60 to 63
years, and it is extremely rare in patients under 40 years of age (11, 12). It has been reported that the survival of young patients with these tumors is poorer than that of their older patient counterparts (13) although the relationship between the clinicopathologic characteristics and age of patients with esophageal adenocarcinoma is not clear. In addition to our knowledge of known predisposing environmental factors, genetic risk factors may also play a significant role in the development of GEJ adenocarcinoma. Characterization of the underlying molecular mechanisms that promote cancer progression could potentially lead to identification of predictive genetic markers that classify patient’s malignant risk. Many studies have identified common genetic alterations associated with a well defined pathological progression from intestinal metaplasia (IM) to low grade dysplasia (LGD), to high grade dysplasia (HGD), and then to carcinoma. Clinical, pathological, and molecular features of GEJ adenocarcinoma in older patients have been extensively studied, but the distribution of these characteristics in the younger population remains unknown. Genes or genetic loci that have been found to be frequently altered include 3p21, 5p15, 5q21-22, EGFR, q36.1, C-myc, p16, p53, Her-2/neu, 20q13.2 and the Y chromosome (14-23). In the recent literature, a high sensitivity and specificity for the detection of dysplasia and esophageal adenocarcinoma was demonstrated by using
multicolor fluorescence in situ hybridization (FISH) DNA probe set specific for the locus specific regions 9p21 (p16), 20q13.2 and Y chromosome. (24).
AIM OF THE STUDY

In an attempt to better understand the causative pathways and to update information on the genetic risk factors related to GEJ adenocarcinoma, we evaluated the clinical features and the role of Y chromosome, the locus 9p21 (p16) loss, and the ploidy of locus 20q13.2 in a spectrum of GEJ mucosa including benign squamous and columnar epithelium, glandular dysplasia, and the invasive carcinoma of patients who underwent surgical treatment under the age of 40 years, and compared their profile with those of more commonly encountered age group (fifth – sixth decades).
METHODS

Patients
We evaluated 663 patients admitted to surgical treatment for GEJ adenocarcinoma and further divided them into 2 age-groups of ≤ 40 and ≥ 50 years, respectively. We identified 29 patients who developed adenocarcinoma under the age of 40 years (mean age of 34.8 ranged from 21–39 years). We retrospectively analyzed and compared the patient’s demographics, tobacco exposure and presence of gastroesophageal reflux disease (GERD) between the two age-groups. In addition, we also examined the esophagectomy technique, the neoadjuvant chemoradiation therapy status and post resection pathologic findings. The pathologic stage was assessed with the 2002 American Joint Commission on Cancer staging system.(25) The disease specific survival was calculated from the date of operation. Patients were followed for the survival through November 30 2006, which constituted the censoring date. The study was performed in accordance with the guidelines of the Institutional Review Board in existence at the time of this analysis.
Operative procedure

Selection of operation was based on identification of the tumor location and depth of tumor invasion and the status of regional lymph node involvement. An Ivor Lewis procedure consisted of a laparotomy and a right thoracotomy with an anastomosis in the chest. A thoracoabdominal procedure consisted of a single left thoracoabdominal incision with an anastomosis in the left chest. A McKeown procedure consisted of a laparotomy, a right thoracotomy, and a left cervical incision, with an anastomosis in the neck. A transhiatal procedure included a laparotomy and a left cervical incision, with an anastomosis in the neck. A transabdominal procedure consisted of a laparotomy only, with an anastomosis above the hiatus and up to the level of the inferior pulmonary vein.
**Material**

Formalin fixed and paraffin embedded tissue sections from surgically resected specimens were examined by gastrointestinal pathologists to select pertinent sections to include the spectrum of histopathological variant of GEJ mucosa for the subsequent investigation by FISH.
Fluorescence in situ hybridization (FISH)

FISH was performed on serial 4-µm tissue sections. The following commercially available fluorescence-labeled, locus-specific (LSI) and centromere DNA probes (CEP) (Vysis Inc, Downers Grove, IL, USA) were applied according to the manufacturer's instructions: LSI 9p21 SpectrumOrange/CEP9 SpectrumGreen, LSI 20q13.2 SpectrumOrange and CEP Y (α satellite) SpectrumOrange.

More than or less than twice the number of red signals than green centromeres signals were scored as a DNA sequence copy number gain or loss for LSI probe, respectively. More than or less than twice the number of green signals were scored as a DNA sequence copy number gain or loss for CEP Y (α satellite) probe, respectively. Digital images were acquired with a Zeiss Axioplan2 imaging microscope (Carl Zeiss Jena GmbH, Jena, Germany) equipped with a PlanApochromat 100×/NA 1.4 oil objective lens and appropriate filter settings for DAPI, SpectrumGreen (FITC), and SpectrumOrange illumination and detection.

FISH with selected DNA probes were investigated and compared within the groups focusing on uninolved squamous and columnar epithelium, intestinal metaplasia, glandular dysplasia, and adenocarcinoma. A minimum of one hundred cells were enumerated per hybridization. Nuclei from normal squamous epithelium or
lymphocytes present on the same slide were used as controls of hybridization efficiency and specificity.
**Statistical analysis**

Statistical analysis was performed using the SPSS statistical package, version 13.0 (Chicago IL) by the $\chi^2$ test and Fisher's exact test for comparison of proportions, and the Mann-Whitney U-test for comparison of non-parametric data. Comparison of means was based on the Student's t-test. Survival was estimated by the Kaplan-Meier method with univariate analysis by the log-rank. Significance was taken at the 5% level.
RESULTS

Clinical and Pathological Features

From the 663 patients admitted to surgery for EGJ adenocarcinoma at our institution there were 539 males and 124 females. The patient’s clinical and pathologic characteristics are summarized in Table 1. There was no significant difference in male to female ratio and presence of symptoms of GERD within the two age groups. The smoking history was more frequently reported in the older patients (72% vs. 48%) p=0.001. Most patients underwent Ivor Lewis esophagectomy without a significant difference regarding the age. Approximately half of the patients in both groups received neoadjuvant chemo or chemo-radio therapy due to their locally advance disease. The two age populations presented with similar pathological characteristics regarding presence of Barrett’s epithelium, glandular dysplasia, and histological differentiation of their tumors. Localized disease (pTNM stage 0, I, and IIA) was present in 62% young patients and 53% older patients (p=0.79), respectively. Survival rate was also similar in the two groups with a three years disease specific survival 54% in patients ≤ 40 years and a 52% in the older ones (p=0.93), respectively.
Table 1

Clinical and pathological characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Mean age</th>
<th>M:F</th>
<th>Tobacco</th>
<th>GERD history</th>
<th>(Path) Barrett's</th>
<th>P Stage 0, I &amp; IIa</th>
<th>Neo Adjuvant</th>
<th>3-Yr DFS</th>
<th>3-Yr OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40 y.o.</td>
<td>29</td>
<td>34.5</td>
<td>23: 6</td>
<td>48.3%</td>
<td>31%</td>
<td>41.4%</td>
<td>62.1%</td>
<td>65.5%</td>
<td>54.1%</td>
<td>64%</td>
</tr>
<tr>
<td>≥ 50 y.o.</td>
<td>634</td>
<td>65.7</td>
<td>516: 118</td>
<td>72.1%</td>
<td>35%</td>
<td>45.7%</td>
<td>52.7%</td>
<td>48.7%</td>
<td>52.2%</td>
<td>54%</td>
</tr>
</tbody>
</table>

*p value*  

|               | <.001 | 0.78  | <.001 | 0.66 | 0.83 | 0.79 | 0.06 | 0.93 | 0.52 |

16
**Chromosomal Aberrations by FISH Analysis**

A total of 28 FISH experiments on 17 patients who were ≤ 40 years and on 11 patients who were ≥ 50 years were performed using LSI 9p21 (p16) SpectrumOrange/CEP9 SpectrumGreen and LSI 20q13.2 SpectrumOrange probes. FISH experiment using CEP Y (α satellite) SpectrumOrange was performed on male patients only (12 patients ≤ 40 years and on 7 patients ≥ 50 years. The control group of 11 patients was randomly selected according to reliably match all the clinical and pathological characteristics of the entire group of patients ≥ 50 years that underwent surgery for EGJ adenocarcinoma.

**Y Chromosome Loss**

Y chromosomal loss has been previously established as a common chromosomal aberration in GEJ adenocarcinoma. In our comparative study between the young and the older patient group, it was apparent that there was a progressive loss of Y chromosome from benign squamous epithelium to Barrett’s mucosa and dysplasia and a near complete loss in adenocarcinoma (90% loss in young and 92% loss in older group) (Table 2 and figure 1A, 1B). While there was a slight trend of more losses during this pathologic progression in older patients, they were not statistically significant different from the young group (Figure 2).
**Table 2**

Average percentage of Y chromosome loss in the different histological categories in the two age groups.

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Normal Squamous</th>
<th>Barrett’s (if present)</th>
<th>Dysplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40 y.o.</td>
<td>12</td>
<td>13.9±7.6</td>
<td>35.2±12.4</td>
<td>70±18.3</td>
<td>90.1±2.19</td>
</tr>
<tr>
<td>≥ 50 y.o.</td>
<td>7</td>
<td>9±2.72</td>
<td>48.7±15.4</td>
<td>86.6±10.1</td>
<td>92.2±5.74</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.273</td>
<td>0.249</td>
<td>0.179</td>
<td>0.360</td>
</tr>
</tbody>
</table>
Figure 1A

Representative examples of squamous cells with biallelic nuclei for Y chromosome (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.
Figure 1B

Representative examples of dysplastic cells with monoallelic nuclei for Y chromosome (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.
Figure 2

Average percentage of cells in GEJ mucosa with normal ploidy or loss for Y chromosome in the different histological categories in the two age groups.
9p21 (p16) Loss

Loss of 9p21 and mutation of the CDKN2/p16 gene have been reported to occur in early lesions during neoplastic progression in Barrett's esophagus. In this study, we demonstrated a significant increase in the percentage of cells showing loss of locus 9p21 during the progression from benign squamous epithelium through dysplasia to adenocarcinoma in both groups (Table 3 and figure 3). The young group revealed significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older group, whereas the percentage of loss was not significantly different in Barrett’s and in the dysplastic epithelium between the two age group (figure 4). The significance in loss 9p21 was not altered regardless the presence of absence of Barrett’s status in each group (Table 4 and figure 5).

In addition, when the young patient group was further stratified into those with the condition of Barrett’s and those without, the average percent of 9p21 loss was significantly higher in benign epithelium (19.7 ± 8.1 vs. 12 ± 2.5, p=0.0158) in the former; and the losses of 9p21 in dysplastic epithelium (51.2 ± 7.2 vs. 53.4 ± 9.4, p=0.6163) and in adenocarcinoma (84.7 ± 14.4 vs. 79.6 ± 3.8, p=0.3208) were similar regardless the Barrett’s status (Table 5 and figure 6).
Table 3

Average percentage of locus 9p21 (p16) loss in the different histological categories in the two age groups.

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Normal Squamous</th>
<th>Normal Columnar</th>
<th>Barrett’s (if present)</th>
<th>Dysplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40 y.o.</td>
<td>17</td>
<td>13.9 ± 5.1</td>
<td>25 ± 6.9</td>
<td>45 ± 6.4</td>
<td>48.5 ± 7.9</td>
<td>81 ± 7.6</td>
</tr>
<tr>
<td>≥ 50 y.o.</td>
<td>11</td>
<td>6.6 ± 4.3</td>
<td>14.6 ± 5.8</td>
<td>55.3 ± 11</td>
<td>46 ± 18.2</td>
<td>39.2 ± 10.5</td>
</tr>
</tbody>
</table>

p value  | 0.0006 | 0.0003 | 0.0042 | 0.6201 | < 0.0001 |
Figure 3

Representative examples of tumor cells with aneuploidy nuclei for locus 20q13.2 (red signal) detected by FISH on formalin fixed and paraffin embedded tissue section.
Figure 4

Average percentage of cells in GEJ mucosa with normal ploidy or loss for locus 9p21 (p16) within the different histological categories in the two age groups. (* = p<0.05)
Table 4

Average percentage of locus 9p21 (p16) losses within the different histologic categories in the two age groups in patients with Barrett’s metaplasia only.

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Normal Squamous</th>
<th>Normal Columnar</th>
<th>Barrett’s Esophagus</th>
<th>Dysplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>8</td>
<td>19.7 ± 8.1</td>
<td>27.9 ± 11.1</td>
<td>45 ± 6.4</td>
<td>51.2 ± 7.2</td>
<td>84.7 ± 14.4</td>
</tr>
<tr>
<td>Old</td>
<td>11</td>
<td>6.6 ± 4.3</td>
<td>14.6 ± 5.8</td>
<td>55.3 ± 11</td>
<td>46 ± 18.2</td>
<td>39.2 ± 10.5</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.0003</td>
<td>0.0033</td>
<td>0.0042</td>
<td>0.4570</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Figure 5

Average percentage of cells in the surgical specimens with normal ploidy or loss for locus 9p21 (p16) within the different histologic categories in the two age groups in patients with Barrett’s metaplasia only. (*) = p<0.05
Table 5

Average percentage of locus 9p21 (p16) losses within the different histologic categories in patients ≤ 40 years with and without Barrett’s metaplasia only.

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Normal Squamous</th>
<th>Normal Columnar</th>
<th>Barrett’s Esophagus</th>
<th>Dysplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Barrett</td>
<td>9</td>
<td>12 ± 2.5</td>
<td>24.1 ± 7.3</td>
<td>-</td>
<td>53.3 ± 9.4</td>
<td>79.6 ± 3.8</td>
</tr>
<tr>
<td>Barrett</td>
<td>8</td>
<td>19.7 ± 8.1</td>
<td>27.9 ± 11.1</td>
<td>45 ± 6.4</td>
<td>51.2 ± 7.2</td>
<td>84.7 ± 14.4</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td><strong>0.0158</strong></td>
<td>0.4121</td>
<td>-</td>
<td>0.6163</td>
<td>0.3208</td>
</tr>
</tbody>
</table>
Figure 6

Average percentage of cells in the surgical specimens with normal ploidy or loss for locus 9p21 (p16) within the different histologic categories in patients ≤ 40 years with and without Barrett’s metaplasia only. (* = p < 0.05)
20q13.2 Gain

In contrast to the loss of Y chromosome and 9p21, the gains of locus 20q13.2 was less significant with progression from benign epithelium through dysplasia to adenocarcinoma (Table 6 and figure 7). A significant difference between the two age group was only detected in the Barrett’s epithelium (young 14.4 ± 4.1 vs. older 11.1 ± 1, p= 0.015) and in a marginal difference in the adenocarcinoma (young 25.0 ± 3.5 vs. older 27.6 ± 2.7, p= 0.0466). Nevertheless both groups presented almost the same trend of progressive gain of 20q13.2 during the dysplastic and neoplastic transformation to carcinoma (Figure 8).
Table 6

Average percentage of locus 20q13.2 gain within the different histologic categories in the two age groups.

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>Normal Squamous (mean±sd)</th>
<th>Normal Columnar (if present)</th>
<th>Barrett’s Dysplasia (if present)</th>
<th>Adenocarcinoma (if present)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40 y.o.</td>
<td>17</td>
<td>0.3 ± 0.4</td>
<td>1 ± 1.2</td>
<td>14.4 ± 4.1</td>
<td>16.6 ± 6.9</td>
</tr>
<tr>
<td>≥ 50 y.o.</td>
<td>11</td>
<td>0</td>
<td>0.5 ± 1</td>
<td>11.1 ± 1</td>
<td>12.2 ± 6.8</td>
</tr>
<tr>
<td>p value</td>
<td>-</td>
<td>0.2621</td>
<td>0.0150</td>
<td>0.1095</td>
<td>0.0466</td>
</tr>
</tbody>
</table>


Figure 7

Representative examples of tumor cells with monoallelic nuclei for p16 (red signal) retaining two copies of chromosome 9 (green signal) detected by FISH on formalin fixed and paraffin embedded tissue section.
Figure 8

Average percentage of cells in GEJ mucosa with gain or loss of locus 20q13.2 within the different histological categories in the two age groups. (* = p<0.05)
DISCUSSION

The cause of the increase in the incidence of adenocarcinoma of the esophagus and gastroesophageal junction in all age groups over the past two decades is unclear. Parallel to the increase in overall incidence, an increase in the number of patients <50 years old as well as those with early intramucosal cancer has been recorded (11, 26). In young patients, the cancer is often diagnosed in a more advanced stage, possibly because of a delay in diagnosis or because of a more biologically aggressive (13).

Our data suggest that, when compared with the older age-group, young patients with GEJ adenocarcinoma possess similar known demographics, environmental factors, clinical and pathologic characteristics. Patients in both groups are predominately males with a history of tobacco exposure, gastroesophageal reflux disease and Barrett’s metaplasia. There was no significant difference in prevalence of early stage disease or disease specific survival when matched for tumor stage. An equal group of patients in each group received neoadjuvant chemoradiation therapy. Thus, our findings do not support the results reported suggesting an higher prevalence of late-stage disease in younger patients at the time of initial diagnosis (11).
FISH and comparative genomic hybridization (CGH) studies have found both gains and losses of chromosomes in the dysplastic and malignant GEJ tissue (16, 23, 24, 27-29).

The most consistent numerical chromosomal aberration found in karyotyping and in similar studies is the loss of the Y chromosome (30, 31). In GEJ adenocarcinoma, Y chromosome loss was found in 31% to 93% of the tumors (32). In one study, the frequency of Y chromosome loss in Barrett’s esophagus increased along with the grade of dysplasia (33). Although Barrett’s associated adenocarcinoma occurs more commonly in men, no specific oncogene or tumor suppressor genes have been assigned to the Y chromosome. It has been proposed that as genetic instability increases during the malignant transformation of Barrett’s mucosa, Y chromosome loss occurs in a random fashion rather than through a specific pathogenic mechanism (32). Evaluation of Y chromosome status by FISH in the sequence normal mucosa – metaplasia – dysplasia – adenocarcinoma has been previously reported by authors (24, 27). Our results confirm the progressive Y chromosome loss in a similar rate in both the young and the older patient populations. Thus the loss of Y chromosome is associated with the development of GEJ adenocarcinoma of male patients in general, but is not restricted to the older age group in particular.
Frequent allelic loss of locus 9p12 and the deletion of corresponding p16 tumor suppressor gene have been reported to occur early in GEJ adenocarcinoma pathway (15, 19), and increase progressively in the metaplasia-dysplasia-carcinoma sequence. The p16 gene encodes a 16-kD protein that forms complexes with the cyclin-dependent kinases CDK4 and CDK6, and subsequently inhibits their ability to phosphorylate the retinoblastoma protein. Unphosphorylated retinoblastoma protein prevents the cell from entering the S phase of the cell cycle. Thus, inactivation of p16 gene may lead to uncontrolled cell growth. Barrett et al. reported a higher prevalence (23%) of p16 gene mutations in GEJ adenocarcinoma with loss of heterozygosity of 9p21 (20). It has been proposed that inactivation of p16 may occur via a number of different molecular mechanisms and p16 inactivation could be a useful biomarker to stratify the risk of progression of Barrett’s metaplasia to dysplasia and neoplasia (34).

In our investigation, the young group reveals significantly more losses of 9p21 in both benign and neoplastic cells when compared to the older group. In addition, the loss was significantly higher in patients with Barrett’s esophagus than those without the condition within the young age group. A significant loss of 9p21 in benign squamous and columnar epithelial cells has not been previously reported. The finding of locus 9p21 genomic loss in majority of dysplasia/adenocarcinoma
cases in this study was consistent with the previous observation that the deletion of p16 gene locus constitutes a major alteration accompanying the progression from Barrett’s esophagus related dysplasia to adenocarcinoma (20, 28, 35). However, the observation of a >80% loss of 9p21 in carcinoma cells in young patients has exceeded the previously reported loss in adenocarcinoma of GEJ in general patient populations (35% - 70%) (24, 27, 36). It is thus plausible that the loss of 9p21 and the subsequent inactivation of p16 in benign appearing and pre-metaplastic epithelial cells constitutes one of the molecular mechanisms that are responsible for the accelerated and early development on adenocarcinoma in young patients.

Chromosome arm 20q was a frequent target of DNA copy gain, usually consisting of gain of the entire chromosome, with a few tumors showing gain of the long arm alone (37).

In particular, 20q13 has been shown to be a frequent site of amplification in breast cancer and amplification of this locus has been associated with immortalization of cells in tissue culture (38). Walch et al. described amplification of locus 20q13.2 by FISH in a subset of patients with esophageal adenocarcinoma (29), and this feature has been confirmed by other studies (39, 40). The most frequently upregulated genes associate with this gain are ZNF217, BCAS1 and CYP24 (41).
Similar to previously reported data, our studies have demonstrated an increase in the percentage of cells showing locus 20q13.2 gain with progression from benign epithelium through dysplasia to adenocarcinoma with almost the same trend in the young and the older group. While a marginal statistical difference are detected in the percentage of nuclei with abnormalities in Barrett’s epithelium and in adenocarcinoma between the two age groups, it is unclear that this difference is responsible for the cancerogenetic process in these patients.
CONCLUSIONS

Patients with GEJ adenocarcinoma who are $\leq 40$ years old share similar clinical and molecular findings with those reported in older patients. Thus GEJ adenocarcinomas in younger are likely to evolve from similar mechanisms of tumor pathogenesis. Possible additional molecular alterations responsible for an eventual accelerated neoplastic process in young patients may include an early and a significant loss and inactivation of p16 gene among other molecular and genetic mechanisms.

Current recommendation regarding indications for endoscopy surveillance in patients with symptomatic reflux have focused on patients over 50-60 years of age (42). Despite advances in endoscopic technology, screening and surveillance strategies for early detection of these tumors have had limited efficacy in preventing these deadly tumors (43). Advances in technologies and molecular diagnosis offer the promise to understand the pathogenesis of this type of adenocarcinoma; among those, FISH may offer a more practical and accurate surveillance tool for patients with GEJ adenocarcinoma. It is clear that young patients with Barrett’s esophagus are not immune to the development of adenocarcinoma and that a liberal use of a molecularly-tailored diagnostic approach may be an appropriate
measure to increase the rate of disease detected at an early curable stage.
REFERENCES


7. Iravani S, Zhang HQ, Yuan ZQ, et al. Modification of insulin-like growth factor 1 receptor, c-Src, and Bcl-XL protein expression
during the progression of Barrett's neoplasia. Human pathology 2003;34: 975-82.


