Loss of imprinting of the insulin-like growth factor II (IGF2) gene in esophageal normal and adenocarcinoma tissues

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To evaluate loss of imprinting (LOI) and expression of the IGF2 gene in esophageal normal and adenocarcinoma tissues, we studied a prospective cohort of 77 patients who underwent esophageal resection between 1998 and 2003. IGF2 imprinting status was determined by reverse transcription–polymerase chain reaction (PCR) following ApaI digestion, and quantitative PCR was used to evaluate IGF2 expression, which was correlated with clinicopathologic findings, disease-free and overall survival. In total, 32% (14/44) of informative tissues showed loss of IGF2 imprinting, with a strong correlation between the tumor and normal esophageal epithelia (Kappa = 0.89, P < 0.01). Normal epithelia with LOI had increased expression of IGF2 [median: 2.91, 95% confidence interval (CI): 0.93–5.06] compared with normally imprinted tumors (median: 1.13, 95% CI: 0.85–1.39) (P = 0.03). In contrast, tumors with LOI had significantly reduced IGF2 expression (median: 1.87, 95% CI: 0.53–5.21) compared with normally imprinted tumors (median: 6.79, 95% CI: 3.39–15.89) (P = 0.016). Patients below the age of 65 with normally imprinted tumors had significantly reduced 5 year disease-free survival (DFS) (24%) compared with patients whose tumors had LOI for IGF2 (55%) (P = 0.03). Cox regression analysis showed that IGF2 overexpression was associated with significantly reduced disease-free survival (P = 0.04). We conclude that in a subgroup of younger patients, loss of IGF2 imprinting was associated with improved outcome following esophageal resection. Expression of IGF2 in esophageal adenocarcinoma and normal esophageal epithelia depended on imprinting status and tissue type, suggesting novel molecular regulatory mechanisms in esophageal tumorigenesis.

Introduction

Over the past three decades, incidence rates for adenocarcinomas of the esophagus and esophagogastric junction (cardia) have increased steadily throughout North America and Europe, exceeding that for any other human solid tumor (1,2). Although the precise reasons for this change are unknown, recent epidemiologic studies have implicated gastroesophageal reflux disease (3) and several lifestyle risk factors, including tobacco consumption, diet and obesity (4–9), in addition to various molecular genetic alterations (reviewed in refs 10,11). As the prognosis for patients with invasive esophageal malignancy remains generally poor (12), it is anticipated that substantial progress in the treatment of this disease will only be made with a clearer understanding of esophageal tumor biology and the incorporation of molecular biomarkers into future clinical practice.

The insulin-like growth factor (IGF) axis is an important regulator of metabolic function, cellular development and growth, and comprises two growth factors (IGF1 and IGF2), several IGF-binding proteins 1–6 and IGF-binding-protein-related proteins, and two principal cell membrane receptors (IGF-IR and IGF-IIR) that are homologous to the insulin receptor (13–15). Recent studies have implicated perturbations of the IGF axis in human malignancy, principally by dysregulation of cellular growth, differentiation and apoptosis, resulting in cellular transformation via paracrine and autocrine mechanisms (16,17). The gene encoding IGF2 is located on chromosome 11p15 and is normally imprinted. Genomic imprinting is an epigenetic modification of a gene, leading to differential expression of the two parental alleles of the gene in somatic cells of the offspring (18). Loss of imprinting (LOI) is therefore defined as the aberrant activation of the normally silent inherited allele, which may result in the expression of a normally silent growth promoting gene, such as IGF2, or the silencing of an expressed growth inhibitory gene on a normally active allele, such as p57KIP2 (19). Although the precise effect of LOI of IGF2 on cell signaling is not well known, LOI has been associated with increased cellular proliferation and an increased sensitivity of the IGF2 signaling pathway (20), in addition to increased risk for gastrointestinal neoplasia and premalignancy (21,22).

Following the initial report of LOI of IGF2 in Wilms’ tumor (23), IGF2 LOI has been reported in several human solid tumors, including malignancies of gastrointestinal tract (24–27). In colorectal cancer, LOI of IGF2 was initially reported in 44% of primary tumor tissues, matched normal colonic epithelia, and peripheral blood lymphocytes (24,25). The finding of a perfect correlation between LOI of IGF2 in circulating lymphocytes, histologically normal colonic mucosa and tumor tissues suggests that IGF2 may have a central role early in multistep colorectal carcinogenesis, possibly by stimulating cell proliferation (24,25). Furthermore, IGF2 LOI has been associated with a distinct colorectal cancer phenotype, comprising onset at a younger age, proximal colon location, poor tumor differentiation and more advanced stage (21,28,29). IGF2 LOI has also been reported to be an independent risk factor for the development of colorectal malignancy (21,30,31).

To date, few studies have evaluated the imprinting status of IGF2 in esophageal malignancy (32). There are currently only two reports of IGF2 LOI in patients with esophageal squamous cell carcinoma, showing the prevalence of IGF2 LOI to range from 54% (7 of 13) to 21% (5 of 24) (26,27). Although IGF2 LOI has not been studied in esophageal adenocarcinoma (EADC), one study evaluated the imprinting status of IGF2 in its premalignant lesion, Barrett’s esophagus, an acquired condition resulting from chronic gastroesophageal reflux disease in which the normal esophageal squamous epithelium is replaced by a specialized metaplastic columnar-cell-lined epithelium. Using endoscopic biopsies of Barrett’s esophagus obtained from 43 patients, the frequency of IGF2 LOI was reported to be 56% (five of nine informative Barrett’s esophagus tissues) (33).

To further define the role of the IGF axis in human esophageal malignancy, the aim of this study was to evaluate IGF2 LOI and expression in a well-characterized series of surgically resected EADC tumors, each with matched histologically normal esophageal epithelium, and to correlate molecular alterations with clinicopathologic findings and post-operative survival.

Materials and methods

This study was based at the Queen Elizabeth II Health Science Centre, Halifax NS, the largest tertiary referral center in Atlantic Canada. All participants gave informed written consent. Approval for banking surgically resected esophageal tissues for molecular studies was obtained from the Department of Pathology, and all tissues were banked according to the 1998 Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans. Approval for this study was granted by the Capital Health Research Ethics Board.
Study subjects
The study population comprised a sequentially accrued series of 77 patients with primary EADC who underwent esophageal resection between February 1998 and December 2003. All patients underwent potentially curative esophagectomy, using a standard surgical approach as previously reported (34). No patient received induction chemotherapy or radiotherapy. Clinicopathologic data and outcome (disease-free survival, DFS—the time interval between surgery and tumor recurrence; overall survival, OS—the time between surgery and last follow-up) were prospectively collected and recorded in a research database. Follow-up was complete for all patients until June 2008.

Esophageal tissues
Immediately following esophageal resection, representative sections of the primary tumor and histologically normal esophageal epithelium adjacent to the proximal resection margin (the site most distant to the primary tumor) were snap-frozen in liquid nitrogen and stored in our esophageal tumor bank at −80°C. All remaining esophageal tissues were processed according to standard protocol and were examined independently by two consultant histopathologists. Tumors were staged according to the International Union Against Cancer classification based on pTNM subsets. Strict clinicopathologic criteria were used to define primary EADCs (Siewert Type I), thereby excluding adenocarcinomas of the cardia (Siewert Type II) or proximal (subcardia) gastric tumors (Siewert Type III).

Extraction of nucleic acids
Genomic DNA and total RNA were extracted simultaneously from banked tissues using the AllPrep DNA/RNA Mini Kit (Qiagen, Mississauga Ontario, Canada) following the manufacturers’ protocol. To avoid the possible DNA contamination, RNA was digested with RNase free DNaseI and cleaned with the RNeasy MiniElute Cleanup Kit (Qiagen).

Imprinting status of IGF2
Based on Apal digestion of a single nucleoside polymorphism on exon 9 of the human IGF2, the imprinting status of IGF2 was determined as previously reported (21,24,35). All PCR products were obtained within the linear range of the reaction. The analysis involved two steps, as follows.

IGF2 genotype. To determine whether samples (tumor and matched normal mucosa) were informative, we used a PCR assay with specific primers for the IGF2 gene: 5′-CTTGGAGTTTGCCATATTGCG (forward) and 5′-GGTCGCGTCATCACTTAC (reverse). The 25 μl PCR reaction was composed of 2 μg genomic DNA, 2.5 μl of 10× PCR buffer, 1.5 mM Mg2+, 0.2 mM deoxy-nucleoside triphosphates, 1 mM each primer and 0.5 U Platinum Taq DNA polymerase (Invitrogen, Burlington, Ontario, Canada). Thermal cycling conditions included an initial denaturation at 94°C for 2 min, with 35 cycles (94°C for 30 s, 55°C for 30 s and 72°C for 1 min), followed by a final extension at 72°C for 10 min. The resulting PCR product (292 bp) was digested with Apal (Invitrogen) at 37°C overnight and electrophoresed in a 2% agarose gel. Samples that were heterozygous for the Apal single nucleoside polymorphism were considered as informative and eligible for further analysis of imprinting status (Figure 1a).

Analysis of allelic expression of IGF2 to determine imprinting status. Complementary DNA (cDNA) was synthesized by reverse transcription (RT) of 2 μg RNA using random primers and SuperScript Reverse Transcriptase III (Invitrogen) according to the manufacturers’ instructions. PCR conditions and primer sequences were as described for IGF2 genotyping (above). The absence of genomic DNA in DNaseI-treated RNA was confirmed by an identical RT minus (RT−) PCR control that lacked the reverse transcriptase. PCR products were digested by Apal and electrophoresed in a 2% agarose gel. LOI of IGF2, biallelic expression, was defined when the ratio of the more abundant allele to the less abundant allele was lower than 3 (Figure 1b), using the Molecular Imager Gel Doc XR System and Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, CA).

Expression of IGF2
Expression levels of IGF2 were determined by RT and quantitative polymerase chain reaction (real time PCR). We selected the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, a housekeeping gene as an internal control, as it was reported to be the least variable gene of 21 housekeeping genes in a previous study of esophageal tissues (36). The real time PCR was performed using the MiniOpticon Real-Time PCR Detection System (Bio-Rad Laboratories) with Taqman Gene Expression Assays and TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA). The assay ID was Hs00171254_m1 for IGF2 (sequence NM_000612.4) and Hs99999905_m1 for GAPDH (sequence NM_002046.3). All assays consisted of a pair of primers and a specific probe labeled with 6-carboxyfluorescein. The PCR reaction (20 μl) was composed of 0.5 μl of cDNA, 1 μl of 20× Taqman Gene Expression Assay and 10 μl of 2× TaqMan Gene Expression Master Mix. The thermal cycles included an initial 50°C for 2 min, 95°C for 10 min and 40 cycles each of 95°C for 15 s, 60°C for 1 min, followed by plate reading. The average cycle threshold (CT) was calculated based on the triplicate assays of each sample. The efficiencies of amplification for IGF2 and GAPDH were determined based on the standard curve created with serially diluted cDNA samples, and were in the range of 92–95%. The logarithm cDNA quantity correlated precisely with the CT value (r2 = 0.990–0.996).

Relative gene expression levels were calculated with 2−ΔΔCT method, which had been validated as described previously (37). Histologically normal gastric mucosa was arbitrarily selected as calibrator. ΔΔCT was calculated by the following formula:

\[
\Delta \Delta CT = (CT_{\text{sample IGF-2}} - CT_{\text{sample GAPDH}}) - (CT_{\text{calibrator IGF-2}} - CT_{\text{calibrator GAPDH}})
\]

Relative IGF2 expression levels were presented as the number of 2−ΔΔCT, which is a fold change of normalized IGF2 expression in samples relative to that in calibrator. The IGF2 expression ratio between tumor and matched normal esophageal epithelia (T/N ratio) was also calculated.

Statistical analysis
Differences in IGF2 imprinting status or expression were compared with a chi-square test, Student’s t-test or Mann–Whitney test and correlations evaluated

![Genotyping and imprinting status analysis of IGF2 gene.](https://academic.oup.com/carcin/article-abstract/30/12/2117/2392145/2118)

**Fig. 1.** Genotyping and imprinting status analysis of IGF2 gene. (a) Gel photo of Apal-digested PCR products for genotyping of IGF2 gene, heterozygous cases were labeled with A/B, which were considered as informative. (b) Gel photo of Apal-digested RT–PCR products for imprinting status analysis. RT− controls for each sample were shown beside the tested samples. Ratios of the more abundant allele to the less abundant allele were listed, indicating one sample had LOI of IGF2.
using Pearson’s linear or Spearman rank tests. The prognostic importance of clinicopathologic variables as well as IGF2 LOI or expression for DFS and OS were compared using Kaplan–Meier survival methods and tested using the logrank test. Multivariate Cox proportional hazards regression methods were used to evaluate the importance of IGF2 LOI or expression on DFS and OS. All analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). Statistical significance was set at $P < 0.05$.

**Results**

**IGF2 imprinting status**

Genotype analysis of DNA samples from the entire series of 77 patients revealed 44 (57%) to be informative. Of these, 14 (32%) tumor and normal tissues showed IGF2 LOI. Only two patients were found to have a difference in imprinting status between tumor and matched normal epithelia (one had LOI in the tumor but normal imprinting in matched normal epithelium, whereas the other had normal imprinting in tumor tissue but LOI in matched normal epithelium) with an otherwise strong correlation of imprinting status between the primary tumor and matched normal esophageal epithelia (Kappa = 0.89, $P < 0.01$).

Selected clinicopathologic features and outcome for the 44 patients with informative tumors are shown in Table I. As expected, predictors of reduced DFS and OS included poor tumor differentiation and advanced tumor stage. No significant differences were found between patient groups whose tumors demonstrated IGF2 LOI ($n = 14$) and those that had normal imprinting status ($n = 30$) with respect to age, gender, tumor differentiation or stage. A trend toward reduced 5 year survival was seen for patients whose tumors were normally imprinted (DFS, 29%; OS, 29%) compared with patients whose tumors exhibited LOI for IGF2 (DFS, 48%; OS, 46%), although this was not statistically significant (DFS, $P = 0.146$; OS, $P = 0.198$). However, when patients below the age of 65 years were considered ($n = 29$) (Table II and Figure 2), significantly reduced 5 year post-operative DFS and OS were compared using Kaplan–Meier survival methods and tested using the logrank test. Multivariate Cox proportional hazards regression methods were used to evaluate the importance of IGF2 LOI or expression on DFS and OS. All analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). Statistical significance was set at $P < 0.05$.

Relative to matched normal esophageal epithelia, 61% (38/62) of primary EADC tumors overexpressed IGF2 (T:N ratio > 2), 10% (6/62) had equivalent expression (T:N ratio = 1–2) and 29% (18/62) underexpressed IGF2 (T:N ratio < 1). IGF2 expression in EADC tumor tissues [median: 5.37, 95% confidence interval (CI): 3.25–8.52] was generally higher than that in normal esophageal epithelia (median: 1.27, 95% CI: 0.99–1.91) ($P < 0.01$). No significant correlation was found between IGF2 expression and tumor stage. However, in patients <65 years, tumors overexpressing IGF2 (relative to matched normal epithelia) were more probably to be poorly differentiated (54%, 14/26 versus 25%, 4/16 tumors with low IGF2 expression) ($P = 0.04$). This observation was confirmed by logistic regression, which showed that increased tumor IGF2 expression was independently associated with poor tumor differentiation (odds ratio 1.20, 95% CI 1.03–1.39; $P = 0.02$). Multivariate Cox regression analysis also showed that IGF2 overexpression was associated with significantly reduced DFS ($P = 0.04$) and OS ($P = 0.06$).

Normal esophageal epithelia with LOI of IGF2 had a 2.9-fold increased expression of IGF2 (median: 2.91, 95% CI: 0.93–5.06) compared with normally imprinted normal esophageal epithelia (median: 1.13, 95% CI: 0.85–1.39) ($P = 0.03$). By contrast, EADC tumor tissues with IGF2 LOI had significantly lower IGF2 expression (median: 1.87, 95% CI: 0.53–5.21) compared with normally imprinted tumors (median: 6.79, 95% CI: 3.39–15.89) ($P = 0.016$).

**Discussion**

The frequency of IGF2 LOI is reported to range from 12 to 100% in various tumors (22), suggesting that aberrant imprinting is an important epigenetic mechanism associated with human carcinogenesis. Using a well-characterized series of primary EADCs, each with matched histologically normal epithelia and detailed clinicopathologic correlative data, we found the prevalence of LOI of IGF2 to be 32% (14 of 44 informative tumors). A strong correlation was found for IGF2 imprinting status between primary tumor and matched normal esophageal epithelia. To our knowledge, this is the first report of LOI of IGF2 in esophageal adenocarcinoma.

Previous clinical and animal studies have demonstrated that the finding of IGF2 LOI in normal colonic mucosa is associated with

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Imprinting status</th>
<th>n</th>
<th>DFS (months)</th>
<th>5 year survival</th>
<th>OS (months)</th>
<th>5 year survival</th>
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<tr>
<td></td>
<td>LOI</td>
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<td>Median</td>
<td>5 year</td>
<td>Median</td>
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<tr>
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<tr>
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<td>18</td>
<td>0.090</td>
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<tr>
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<td>17</td>
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<td>0.883</td>
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<td>19</td>
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<tr>
<td>&gt;65</td>
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<td>11</td>
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<td>46</td>
<td>0.199</td>
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<td>9</td>
<td>0.991</td>
<td>14</td>
<td>0.758</td>
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<td>0.0001</td>
<td>6</td>
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*Tumors were staged according to the International Union Against Cancer classification based on pTNM subsets.*
an increased frequency of premalignant adenomas compared with normally imprinted tissues (24,30,38). Furthermore, mice with LOI of IGF2 were also found to have generally less differentiated colonic epithelium, reflected by longer intestinal crypts and increased immunohistochemical staining for progenitor cell markers, and with the subsequent development of increased numbers of colonic tumors compared with normally imprinted controls (38). Although it was proposed that altered maturation of non-neoplastic tissues may be one mechanism by which epigenetic changes affect cancer risk, the significance of IGF2 LOI in normal esophageal epithelia remains unclear.

Of particular interest was our finding that genomic imprinting was associated with adverse outcome in a subset of younger patients (<65 years) with EADC. The importance of age has previously been recognized in studies evaluating IGF2 LOI in colorectal malignancy, where a distinct tumor phenotype was identified (21,28,29). In our study of surgically resected primary EADCs, reduced post-operative survival was found to be associated with poor tumor differentiation and advanced tumor stage, as expected (Table I). However, we found that patients below the age of 65 years, whose tumors exhibited normal genomic imprinting for IGF2, had significantly reduced post-operative DFS compared with patients whose tumors were found to have LOI (Figure 2). No significant differences with respect to age, gender, tumor differentiation or stage were found between patient groups to account for survival differences. These findings suggest distinct carcinogenic pathways for EADC based on IGF2 genomic

![Disease Free Survival](image)

**Fig. 2.** Survival curves for 29 patients <65 years stratified by IGF2 imprinting status. Five year DFS (upper) was significantly higher for 11 patients whose tumors were found to have LOI of IGF2 (55%), compared with 18 patients with normally imprinted tumors (5 year DFS, 24%) \( (P = 0.03) \). A similar trend was seen for OS at 5 years (52% 5 year OS for LOI versus 24% if normally imprinted), but statistical significance was not reached \( (P = 0.07) \).
imprinting status, which may have implications for screening, prognosis and therapy.

Overexpression of IGF2 has been reported in various solid tumors, including colorectal cancer (38-43) and esophageal squamous cell carcinoma (26,27). As IGF2 expression has not been studied, to date, in EADC, we evaluated a subset of 68 patients from whose banked esophageal tissues usable RNA was successfully extracted for expression analysis. Here, we found that IGF2 was significantly overexpressed in primary EADC tumor tissues compared with matched normal esophageal epithelia. In patients <65 years, IGF2 overexpression was associated with poor tumor differentiation. A similar association between IGF2 overexpression and poor tumor differentiation has also been reported for esophageal squamous cell carcinoma (27).

In keeping with previous studies of colorectal cancer (42,44), we also found that patients whose tumors overexpressed IGF2 had significantly reduced post-operative survival, independent of tumor differentiation or stage.

Although the underlying molecular mechanisms resulting in IGF2 overexpression are unknown, recent results from both animal and clinical studies have implicated genomic imprinting as a potential mechanism modulating IGF2 expression (20,30,38). In this study, we demonstrated that histologically normal esophageal epithelia with LOI had >2-fold expression of IGF2 compared with normally imprinted epithelia, suggesting that the activation of the normally silenced maternal allele of the IGF2 gene may be responsible for IGF2 overexpression in normal tissues. Surprisingly, we found that EADC tumors with LOI of IGF2 expressed significantly lower levels of IGF2 than normally imprinted tumors. However, this observation was in keeping with our finding of improved post-operative survival seen for patients whose primary EADC tumors exhibited IGF2 LOI (Figure 2), most probably reflecting lower levels of tumor IGF2 expression.

The paradoxical expression of IGF2 by esophageal normal and tumor tissues exhibiting LOI of IGF2 raises important questions as to the molecular mechanisms and role of IGF2 in esophageal tumorigenesis. It is conceivable that IGF2 may well have a dual role, dependent on both the temporal sequence of multistep carcinogenesis and the tissue specificity. The importance of LOI of IGF2 in normal gastrointestinal epithelia with respect to increased risk of colorectal preneoplastic and neoplastic lesions has been identified (30,38), and our finding that normal esophageal epithelia with LOI have increased levels of IGF2 expression compared with normally imprinted tissues would suggest a central role for IGF2 LOI at an early stage of esophageal tumorigenesis. This is also supported by the recently proposed epigenetic progenitor model, indicating LOI may play important role in progenitor cells rather than in established tumors (45). Although the resulting overexpression of IGF2 in normal esophageal epithelia may well result in increased cellular proliferation, decreased apoptosis or otherwise altered cell kinetics in normal esophageal epithelia, further studies will be required to precisely define potential downstream targets, pathways and interactions.

That EADC tumor tissues with LOI of IGF2 have lower levels of IGF2 expression relative to normally imprinted tumors suggests alternate, tissue-specific, molecular regulatory mechanisms. Expression of IGF2 has also been reported to be induced by mutations in its downstream regulatory 3′ untranslated region (46), by altered methylation of a differentially methylated region upstream of the H19 promoter (35,47), and recently by natural antisense transcription (48,49). In addition, allelotype analyses of primary EADC tumors have demonstrated a high frequency of allelic losses of chromosome 11p, which may further modulate gene expression (50). As IGF2 signaling is mediated by the IGF-1 receptor, we also evaluated IGF1R expression in esophageal tissues (51) and found a significant correlation in both EADC (rs = 0.47, P < 0.01) and matched normal epithelia (rs = 0.46, P < 0.01), suggesting potential autocrine regulatory mechanisms.

Despite the striking changes reported recently for the epidemiology of esophageal cancer (1,2), EADC is a relatively uncommon tumor in North America, with incidence rates generally <10 per 100 000 population. Given the rarity of this malignancy, this consecutive series of tumors, each with matched normal epithelia, would be considered a relatively large surgical series accrued over a 70 month interval. The distribution of tumor stage and predictors of survival (Table I and II) further suggest that this series is representative of esophageal malignancy. Additional strengths of this study include that all tumors were treated in a consistent manner by a single university-based surgeon using a consistent operative approach (34) and that no patient received preoperative chemotheraphy or radiation therapy, which is now routine at many centers in North America and Europe and which may potentially confound molecular studies. Furthermore, all tumors were well staged pathologically, with strict clinicopathologic criteria to define primary EADCs, thereby avoiding misclassification with adenocarcinomas of the gastric cardia, and follow-up and outcomes data were complete for all patients.

In summary, this is the largest and most comprehensive study of IGF2 overexpression in primary EADC tumors and matched normal esophageal tissues. We have demonstrated the prevalence of LOI of IGF2 to be 32% (14 of 44 informative esophageal tissues), suggesting IGF2 LOI may be a clinically relevant molecular marker for EADC. We have also demonstrated that in a subgroup of younger patients, imprinting status is associated with post-operative outcome following esophageal resection. Further characterization of IGF2 expression in primary EADC tumor and matched normal epithelia has shown that IGF2 overexpression depends on imprinting status and tissue type, suggesting novel molecular regulatory mechanisms in esophageal tumorigenesis.

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Conflict of Interest statement: None declared.

References


Table III. Selected clinicopathologic factors and outcome following surgical resection of esophageal adenocarcinoma for 68 patients whose tissues were evaluated for IGF2 expression

<table>
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<tr>
<th>Classification</th>
<th>n (%)</th>
<th>DFS (%)</th>
<th>P</th>
<th>OS (%)</th>
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*Tumors were staged according to the International Union Against Cancer classification based on pTNM subsets.

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