IGF axis gene expression patterns are prognostic of survival in epithelial ovarian cancer

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Abstract

The IGF axis has documented growth-promoting effects in various malignancies, but its role in epithelial ovarian cancer (EOC) has not been adequately examined. We studied the expression of the IGF axis genes in relation to outcome in EOC. Microarray expression profiles from 64 patients with advanced-stage EOC were used. Two multi-gene subsets were chosen, one upstream of the IGF receptor (‘IGF family’) and the other downstream of the IGF receptor (‘IGF signaling pathway’), and analyzed in relation to survival. In addition, expression patterns of the two gene subsets were analyzed in relation to favorable and unfavorable prognosis categories identified in a previous study by whole-genome expression profiling. In a gene-by-gene analysis, IGF binding protein 4 and IGF-II receptor gene expression was inversely associated with survival. Using hierarchical clustering, the two multi-gene subsets separated the patient cohort into two groups with different median survival (IGF family: 33 vs 63 months, \( P = 0.02 \) and IGF signaling pathway: 41 vs 63 months, \( P = 0.05 \)). Furthermore, the two multi-gene subsets were differentially expressed between the previously defined favorable and unfavorable prognosis tumors (Kolmogorov–Smirnov permutation: \( P = 0.0005 \) and 0.003 for the IGF family and signaling pathway respectively), and individual genes (including IGF-I, IGF-I receptor, and several genes downstream of the receptor) were overexpressed in unfavorable prognosis tumors (permutation \( P < 0.05 \)). The expression patterns of several genes in the IGF axis are associated with survival in EOC, and expression changes of these genes may be underlying previously proposed microarray-derived clinical prognostic models. Future studies are needed to more precisely determine the diagnostic and potential therapeutic significance of these findings.

Introduction

Insulin-like growth factors (IGFs) are a group of peptide growth factors (IGF-I and IGF-II) sharing structural homologies and functional properties with insulin (Stewart & Rotwein 1996). Together with their binding proteins (IGFBPs), receptors, and downstream signaling molecules, circulating growth factors constitute an endocrine system (usually referred to as the ‘IGF axis’) regulating metabolism and growth (Stewart & Rotwein 1996). In addition, paracrine and autocrine circuits involving IGFs have also been described (Yee et al. 1989). Thus, a great interest has been generated in studying the potential role of the IGF axis in cancer biology. In vitro models provide support for a growth-promoting and anti-apoptotic effect of IGF-I in various malignant cell types (LeRoith & Roberts 2003, Pollak et al. 2004), and clinical studies have established associations between serum levels of IGF-I or specific IGFBPs and risk for, or progression of various epithelial cancers including prostate, colon, and breast cancer (Mantzoros et al. 1997, Chan et al. 2000, Giovannucci et al. 2000, Hankinson & Schernhammer 2003, LeRoith...


& Roberts 2003). Finally, recent reports have highlighted the therapeutic potential of IGF-I receptor inhibition, in vitro and in vivo, for certain malignancies (Min et al. 2005, Mitsiades & Mitsiades 2005).

In contrast to other epithelial malignancies, limited data are available on the potential role of the IGF axis in epithelial ovarian cancer (EOC). In vitro models demonstrate that EOC cell lines express all major components of the IGF family, that exogenous IGF administration induces cell proliferation (Conover et al. 1998, Kalli et al. 2002), and that IGF-I and IGFBP2 promote ovarian cancer cell growth and invasiveness (Kalli & Conover 2003, Chakrabarty & Kondratick 2006). In addition, elevated serum levels of IGF-I and IGFBP2 have been associated with an increased risk of ovarian cancer (Lukanova et al. 2002, Dal Maso et al. 2004), and in one recent study, IGF-II expression was shown to be upregulated in the ovarian tumor tissue relative to normal ovarian surface epithelium (Sayer et al. 2005). While in this study IGF-II overexpression was also prognostic of poor outcome (Sayer et al. 2005), no previous reports have assessed other members of the IGF axis and/or performed a comprehensive expression analysis in relation to outcome using primary ovarian cancer tissue. Microarray technology allows the study of expression patterns of gene families and pathways allowing for the possibility that no single molecule may adequately capture the role of a gene family or pathway in malignant pathogenesis and progression. Thus, we used microarray profiles to determine whether expression patterns of the genes of the IGF axis, either individually or collectively, may have prognostic significance in this highly lethal disease.

Materials and methods

Patients and tumor samples

The study population consisted of 68 patients with advanced (International Federation of Gynecology and Obstetrics, FIGO stage III/IV) EOC from Beth Israel Deaconess Medical Center (BIDMC) and Memorial Sloan Kettering Cancer Center (MSKCC), diagnosed between 1995 and 2000. The characteristics of the patient cohort have been described previously (Spentzos et al. 2004, 2005). All patients had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy according to standard debulking guidelines by an expert gynecologic oncologist, and all received standard adjuvant platinum/taxane-based chemotherapy (Cannistra 2004). Follow-up data for this study were derived from the Ovarian Cancer Relational Database at BIDMC and the Ovarian Cancer Clinical Database at MSKCC. Tissue and clinical data collection was approved by the Institutional Review Boards at both Institutions and all patients provided informed consent. Ovarian cancer samples were collected at the time of primary debulking surgery and frozen at −80 °C. Non-microdissected tumor specimens were used in order to maintain the ability to capture the role of the stroma and potential paracrine IGF loops.

Clinical definitions

Staging was reported according to the FIGO (Cannistra 2004). Optimal debulking was defined as ≤ 1 cm gross residual disease, and suboptimal debulking as more than 1 cm residual disease. Overall survival (OS) was defined as the time between the date of diagnosis and the date of death from ovarian cancer. OS information used in the previously published study was updated as of January 2007, for the purposes of this study.

RNA isolation, cDNA synthesis, microarray probe preparation, and Affymetrix GeneChip hybridisation

These procedures were performed as per standard manufacturer protocols and have been extensively reported in previous publications (Spentzos et al. 2004, 2005). Tumor samples were pulverized and homogenized in liquid nitrogen and RNA was isolated using the Trizol method. The Affymetrix U95A2 array containing 12 625 transcripts was used for hybridization of labeled RNA probes as described previously (Spentzos et al. 2004, 2005). Image analysis and signal processing were carried out using the dChip algorithm and two arrays were excluded from further analysis because they were determined to be technical outliers as per the dChip criteria (Li & Wong 2001a, b). The microarray dataset and all technical parameters have been reported in the context of a previously published study with a different research focus (Spentzos et al. 2005).

‘IGF family’ and ‘IGF signaling pathway’ gene expression matrix extraction

A computerized search of the U95A2 array revealed 42 probe sets corresponding to genes whose name contains the terms ‘insulin’, ‘insulin-like’, or ‘IGF’. These transcripts included the two growth factors (IGF-I and IGF-II), their receptors, insulin receptor substrate (IRS1), as well as several IGFBP1-7. After examining the expression calls on the Affymetrix arrays, 12 probe sets were excluded from further
analysis because they produced an ‘absent’ signal in all samples; the remaining 28 probe sets, representing genes upstream of the IGF receptor, were called ‘IGF family’. An additional set of genes was retrieved from the publicly available Biocarta Pathway database of the NCI Cancer Genome Anatomy Project (CGAP) and mapped on to the U95A2 array (37 probe sets). This gene set has been independently characterized by the CGAP as an ‘IGF signaling pathway’ representing components of the IGF axis whose gene products participate in intracellular signaling downstream of the IGF receptor. These two probe-set groups (‘IGF family’ and ‘IGF signaling pathway’) comprise the autocrine/paracrine IGF axis and together with the associated expression data form the basis of this study. Tables with all the names of the genes included in the IGF family and pathway lists and the associated expression data are provided in the on-line supplement (www.bidmcgenomics.org/ovcaigf/).

Bioinformatics and statistical analysis

Algorithms implemented in the NCI BRB Array Tools software package (developed by Richard Simon and Amy Peng Lam) were used for statistical analyses (Wright & Simon 2003, Jazaeri et al. 2005, Spentzos et al. 2005). Expression values were bottom-filtered at 10, according to default software parameters. The differences in the expression of individual genes among categorical disease phenotypes were assessed by a log-transformed t-test, and statistical significance, corrected for multiple testing, was assessed by a multivariate random permutation test as described previously (Wright & Simon 2003, Jazaeri et al. 2005) limiting the false discovery rate to 10%. Enrichment and differential expression of multi-gene sets (‘IGF family’ and ‘IGF signaling pathway’ taken as potential functional entities) between sample groups was analyzed by the functional scoring method which was also implemented in the NCI BRB Array tools package, testing the null hypothesis that the total number of significant family or pathway genes is a random collection from the bigger list of differentially expressed genes. This method applies a Kolmogorov–Smirnov (KS) test, assessing the distribution of a pre-defined gene set (‘IGF family’ or ‘IGF signaling pathway’) expression, within a large ranked gene list consisting of all genes differentially expressed between two sample groups (Pavlidis et al. 2002). Hierarchical clustering was implemented using standard approaches as described previously (Eisen et al. 1998). The correlation between continuous variables was assessed by the Spearman correlation coefficient, and associations between continuous and categorical variables were assessed by the Mann–Whitney test. Associations between expression variables and survival were assessed by the Cox Proportional Hazards method (CPH, SPSS 11.0 package) with the use of clinical categorical covariates as appropriate.

Real-time PCR

Reverse transcription was performed using 1 μg starting total RNA and the Promega Reverse Transcription System (Promega) according to the manufacturer’s instructions. Real-time PCR was carried out using the SYBR Green I-based real-time PCR on the MJ Research DNA Engine Opticon Continuous Fluorescence Detection System (MJ Research Inc., Waltham, MA, USA), as described previously (Zerbini et al. 2003, 2004, 2006, Gu et al. 2007). We performed real-time PCR for select genes on 43 samples for which RNA was still available from our original microarray experiments, and then analyzed the results for their correlation with microarray expression values for these samples and the fold changes between poor and good prognosis tumors. Detailed methods and primer sequences are provided in the on-line supplement (www.bidmcgenomics.org/ovcaigf/).

Results

Clinical and pathological characteristics

The clinical and pathological characteristics of the patients (Table 1) have been published previously (Spentzos et al. 2004, 2005). The median patient age was 57 years. All patients had advanced-stage disease,

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>57 (36–80)</td>
</tr>
<tr>
<td>Stage (FIGO)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>57 (89%)</td>
</tr>
<tr>
<td>IV</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>Debulking status</td>
<td></td>
</tr>
<tr>
<td>Optimal (≤ 1 cm)</td>
<td>40 (63%)</td>
</tr>
<tr>
<td>Suboptimal (&gt; 1 cm)</td>
<td>24 (37%)</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
</tr>
<tr>
<td>Serous (pure or mixed)</td>
<td>58 (91%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>5 (7.5%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13 (29%)</td>
</tr>
<tr>
<td>3</td>
<td>51 (80%)</td>
</tr>
</tbody>
</table>

*All patients received standard adjuvant platinum/taxane-based chemotherapy.
and the majority had grade 3 (80%), serous (91%) EOC. After primary surgery, 63% of patients were optimally debulked. Survival information initially used for previous publications was updated for the purposes of this study. As of January 2007, 18 patients from the entire cohort remain alive and all the rest have died of cancer.

**IGF family gene correlations**

While associations between serum levels of various IGF family proteins have been described previously, limited data have been reported on the associations between IGF family gene transcript levels in ovarian and other tumor tissues. Using Spearman’s correlation coefficients ($r$), we observed the following significant correlations ($P < 0.05$): 1) IGF-I was correlated with IGF-II receptor ($r = 0.25$), IGFBP1 ($r = -0.22$), IGFBP2 ($r = 0.55$), IGFBP3 ($r = 0.38$), and IGFBP4 ($r = 0.61$); 2) IGF-II was correlated with the IGF-II receptor ($r = 0.25$), IGFBP2 ($r = 0.43$), and IGFBP3 ($r = 0.27$); 3) IGF-I receptor was correlated with IGFBP2 ($r = 0.23$), IRS1 ($r = 0.48$), and insulin-induced gene 2 ($r = 0.37$); 4) IGF-II receptor was also correlated with IGFBP2 ($r = 0.46$) and IGFBP3 ($r = 0.3$), and IGFBP3 with IGFBP4 ($r = 0.56$).

**Global assessment of IGF family association with survival**

Given the significant correlations that were observed in expression levels among the various IGF family genes, and their known biological interactions, we hypothesized that collective assessment of the gene expression of the entire family may offer information relevant to outcome. Therefore, we first analyzed the expression pattern of the IGF gene family as a single entity by performing unsupervised hierarchical clustering of all ovarian tumor samples as a function of the expression patterns of all the 30 probe sets simultaneously. This analysis revealed two predominant groups of patients with significantly different survival curves (median survival 33 vs 63 months, log rank $P = 0.02$, Fig. 1A). The Hazard ratio (HR) for the poor prognosis versus the good prognosis group was 2.1 (95% CI: 1.2–3.9) by CPH Model.

**Univariate associations between IGF family gene expression levels and survival**

We then used Cox regression analysis to further explore the expression of individual IGF family genes in relation to ovarian cancer outcome. IGFBP4 and IGF-II receptor gene expression was inversely associated with survival (HR 1.51, $P = 0.03$ and HR 4.0, $P = 0.04$ for a twofold increase in gene expression respectively), while IRS2 gene expression was positively associated with survival (HR 0.31, $P = 0.015$) as well.

**Multivariate analysis of the association between IGF family gene expression levels and survival**

Our study cohort consisted of advanced-stage tumors which were of predominantly high grade and serous histology (Table 1). None of the IGF family genes found to be significantly associated with survival in univariate analysis was associated with other known clinical confounders including age (analyzed as either a continuous or a categorical variable with a cut-off at 60 years), grade (analyzed as low versus high), and debulking status. In a multivariate CPH model including age, debulking status, and grade as covariates, the association of IGFBP4, IGF-II receptor, and IRS2 with survival remained unchanged (HR 1.5, $P = 0.02$; HR 4.0, $P = 0.06$; and HR 0.3, $P = 0.005$ for a twofold increase in expression levels respectively).
Expression patterns of downstream IGF pathway genes in relation to ovarian cancer survival

In order to further explore the relation of the IGF axis with ovarian cancer outcome, we also examined the expression patterns of the IGF signaling pathway genes in relation to survival, utilizing another gene set that has been independently characterized as an IGF signaling pathway by the NCI CGAP (Fig. 2). We mapped the pathway genes onto the Affymetrix GeneChip probe sets and performed hierarchical clustering using the 42 IGF signaling pathway probe sets. This analysis separated the patient cohort into two main groups, which again showed significant survival differences (median OS 41 vs 63 months, log rank $P = 0.05$, Fig. 1B) demonstrating that the IGF signaling pathway gene expression also contains information relevant to ovarian cancer survival. The HR for the poor prognosis versus the good prognosis group was 1.9 (95% CI: 1.01–3.2).

Expression patterns of IGF axis genes as a function of previously established prognostic categories by gene profiling

We recently described a gene expression profile (Ovarian Cancer Prognostic Profile-OCPP) that was a strong prognostic factor for OS in EOC using the same patient population. This profile consisted of 115 Affymetrix probe sets and discriminated between groups of patients with favorable and unfavorable prognosis (Spentzos et al. 2004). We were thus interested to study the expression patterns of the IGF family genes in relation to the prognostic categories defined by the OCPP. We first assessed the entire group of genes as a single functional entity and found that there are significant global expression differences between the favorable and the unfavorable prognostic categories as previously defined by the OCPP (KS permutation $P = 0.0005$). We then assessed the expression patterns of individual genes within the IGF family and also found expression changes in several genes between the two prognostic categories (Table 2). Specifically, IGF-I showed a 4.7-fold overexpression in the unfavorable prognosis group ($P = 0.00004$ or $<0.0001$ from a random permutation test and with a false discovery rate of 10% allowed), while a number of other genes (including IGF-I receptor and various binding proteins) also showed significant overexpression in the unfavorable group. Finally, to examine whether any of these genes could serve as markers with prognostic information additional to that of the OCPP, we carried out multivariate Cox regression analyses, including the OCPP and each of the univariately significant IGF

Figure 2 IGF-I signaling pathway (from the NCI Cancer Genome Anatomy Project, Biocarta database, Genes differentially expressed ($P < 0.05$, FDR 10%) between the two OCPP-derived prognostic categories are marked with a white circle. (http://www.biocarta.com/pathfiles/h_igf1Pathway.asp). Reproduced with permission, copyright BioCarta, Inc. 2007.

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When the 42 probe sets representing the previously established prognostic categories by OCPP.

IGF

survival is already reflected in the networks captured from the original microarray analysis. Despite the using 43 samples for which RNA was still available by microarray analysis, we performed real-time PCR in order to validate the expression patterns discovered in this study, the essentially identical to that described in the previous work.

family genes as covariates. While the association between the OCPP and survival remained strong and essentially identical to that described in the previous study, the IGF family genes were not independently associated with survival in this analysis, suggesting that the biological link of IGF family genes with survival is already reflected in the networks captured by the OCPP signature.

We then also examined the expression patterns of the IGF signaling pathway genes as a function of the previously established prognostic categories by OCPP. When the 42 probe sets representing the IGF-I pathway genes were assessed as a single entity, the IGF signaling pathway showed significant global expression differences (KS permutation \( P = 0.003 \)) between the two prognostic groups (favorable and unfavorable). Subsequently, we tested individual genes within the pathway and found that many of them were differentially expressed between the two prognostic groups at \( P < 0.05 \) (by random permutation test and controlling for multiple comparisons with a false discovery rate of 10%) as shown in Table 3.

The expression patterns of all IGF axis genes, upstream and downstream of the receptor, as a function of the OCPP-derived prognostic categories are displayed in a colored heatmap (available online at www.bidmcgeromics.org/ovcaigf/), showing that coordinated overexpression of many IGF axis genes is a feature of the poor prognosis tumors.

### Real-time PCR

In order to validate the expression patterns discovered by microarray analysis, we performed real-time PCR using 43 samples for which RNA was still available from the original microarray analysis. Despite the smaller subset of samples available, fold expression ratios between poor prognosis and good prognosis tumors, as defined in the previous paragraph, were of a direction and magnitude similar to the expression ratios previously observed by microarray analysis for the same genes, described in Tables 2 and 3 (IGFBP2: 2.0, FOS: 5, IGFBP4: 3.1, IGFBP7, 1.5, IRS1 4.9). In addition, significant Spearman’s correlations (\( r > 0.4, P < 0.01 \)) were observed between the expression levels obtained by microarray and those obtained by real-time PCR for the subset of the 43 samples for IGF-I, IGFBP2, IGFBP7, and IRS1.

### Discussion

Several genes of the IGF axis have been shown to be associated with an increased risk for and progression of various malignancies, but the role of the IGF axis in EOC has not yet been fully elucidated (Moschos & Mantzoros 2002, LeRoith & Roberts 2003); most of the available information derives from in vitro work. In this study, we used primary tumor microarray profiles to comprehensively analyze tissue expression of the genes of the entire IGF axis in a cohort of patients with advanced-stage ovarian cancer. Our analysis included two sets of genes, one upstream of the IGF receptor comprised the ligands, receptors, and binding proteins (“IGF family”) and the other downstream of the IGF

### Table 2 Expression of the Insulin-like growth factor (IGF) family genes in relation to previously described prognostic categories by whole-genome expression profiling\(^a, b\)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Average fold difference (unfavorable/favorable group)(^a)</th>
<th>( P ) value(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>4.7</td>
<td>0.000 006</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>1.8</td>
<td>0.008</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>1.7</td>
<td>0.02</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>2.7</td>
<td>&lt;0.000 002</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>2.0</td>
<td>0.0009</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>1.8</td>
<td>0.001</td>
</tr>
<tr>
<td>IGFBP7</td>
<td>2.3</td>
<td>0.000 004</td>
</tr>
</tbody>
</table>

\(^a\)Prognostic categories described in previous work (Spentzos et al. 2004).

\(^b\)Genes NOT differentially expressed between different prognosis tumors: IGF-II, IGFBP1, insulin, insulin-induced gene 1, insulin degrading enzyme.

\(^c\)By permutation \( t \) test. False discovery rate (FDR), 10%.

### Table 3 Expression of the Insulin-like growth factor (IGF) pathway genes in relation to previously described prognostic categories by whole-genome expression profiling\(^a\)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Average fold difference (unfavorable/favorable group)(^a)</th>
<th>( P ) value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRS1</td>
<td>2.7</td>
<td>0.000 002</td>
</tr>
<tr>
<td>FOS</td>
<td>4.8</td>
<td>0.000 003</td>
</tr>
<tr>
<td>JUN</td>
<td>2.6</td>
<td>0.000 006</td>
</tr>
<tr>
<td>Insulin-induced gene 2</td>
<td>1.7</td>
<td>0.000 006</td>
</tr>
<tr>
<td>SHC1</td>
<td>1.6</td>
<td>0.00001</td>
</tr>
<tr>
<td>SOS1</td>
<td>1.7</td>
<td>0.00001</td>
</tr>
<tr>
<td>RASA1</td>
<td>1.7</td>
<td>0.00001</td>
</tr>
<tr>
<td>PTPN11</td>
<td>1.7</td>
<td>0.0003</td>
</tr>
<tr>
<td>IGF-I receptor</td>
<td>1.4</td>
<td>0.006</td>
</tr>
<tr>
<td>CSNK2A1</td>
<td>1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>SRF</td>
<td>1.2</td>
<td>0.02</td>
</tr>
<tr>
<td>ELK1</td>
<td>0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>RAF1</td>
<td>1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>GRB2</td>
<td>0.8</td>
<td>0.03</td>
</tr>
</tbody>
</table>

## Genes NOT differentially expressed between different prognosis tumors: IGF-II receptor, IRS2, insulin-induced gene 1, MAPK3, MAP2K1, HRAS, PTPN11, MAPK8.

\(^a\)Prognostic categories described in previous work (Spentzos et al. 2004).

\(^b\)By permutation \( t \) test. False discovery rate (FDR), 10%.
receptor comprised several signaling molecules (‘IGF signaling pathway’). Our findings demonstrate that expression patterns of the IGF axis genes are associated with survival and that overexpression of several key members of this axis is associated with poor prognosis in EOC.

We observed significant correlations between expression levels of various IGF axis genes, suggesting that their effects on the disease may be linked. Indeed, using the expression patterns of all genes of the IGF family we observed a significant association with survival as demonstrated by the clustering of the patient cohort into two groups with median OS of 33 and 63 months respectively (Fig. 1A). In addition to the effect of the IGF family as a whole, individual genes within the family were also associated with outcome. Specifically, expression of the IGFBP4 and IGF receptor 2 genes was inversely associated with survival and expression of IRS2 was positively associated with survival even when controlled for other clinical prognostic factors. While the precise biologic role of these genes in ovarian cancer remains to be investigated, a previous in vitro report suggested that IGFBP4 prevents breast cancer cell apoptosis, a role compatible with the expression pattern observed in our dataset (Perks et al. 1999). On the other hand, the IGF-II receptor facilitates internalization/degradation of the IGF-II ligand (O’Dell & Day 1998), while the IRS2 is thought to modulate IGF signaling. Thus, it is conceivable that IGF-II receptor and IRS2 expression changes are compensatory to and possibly a marker of increased IGF activity. Irrespective of these specific observations, the clear association of the IGF family as a whole with survival is consistent with the notion, common in genomic profiling studies, that deregulations in multiple genes, some of which may be subtle and not individually significant by traditional statistical criteria, may collectively contribute to the effect of the pathway on tumor behavior (Hosack et al. 2003, Lamb et al. 2003, Subramanian et al. 2005).

Further support for the possible relevance of the IGF axis to EOC outcome was obtained by our finding that the expression patterns of the signaling molecules downstream of the IGF receptor were also associated with survival. Thus, clustering the patient cohort using a set of genes previously curated by the NIH CGAP and designated as the ‘IGF-I signaling pathway’ (Fig. 2) resulted in two groups with median survival of 41 vs 63 months (Fig. 1B), indicating that expression patterns of both upstream and downstream components of the IGF axis convey information relevant to survival in EOC.

Utilizing the same patient cohort, we have previously described and validated a gene signature called Ovarian Cancer Prognostic Profile (‘OCPP’) offering strong independent prognostic information for EOC (Spentzos et al. 2004). Diagnostic and prognostic molecular profiles geared towards clinical prediction consist, by design, of a relatively small number of genes with best performance as markers and do not represent an exhaustive list of all molecular differences between different tumor phenotypes. Although the IGF family genes were not included in the OCPP and did not appear to add to its prognostic power from a statistical point of view, we were interested in examining whether deregulation in IGF family gene expression might nonetheless be one of the biological processes underlying and reflected in the OCPP-derived prognostic classification. Indeed, significant difference was discovered when we compared the expression patterns of the IGF family as a multi-gene entity between tumors with favorable and unfavorable prognosis as defined by the OCPP. Moreover, many individual genes, including IGF-I and IGF-I receptor, showed several-fold upregulation in the OCPP-defined unfavorable prognosis patient category (Table 2) in a gene-by-gene analysis corrected for multiple testing, indicating that the relation of the IGF family genes with outcome can be better appreciated when analyzed in the context of the OCPP prognostic categories.

These findings are compatible with several in vitro reports that have proposed functional links between members of the IGF family and specific genes contained in the OCPP. For example, both IGF-I and IGF-II are known to increase expression of fibronectin (FN), the overexpression of which was a marker of poor prognosis in our previous study (Pricci et al. 1996). In turn, FN has been shown to upregulate and promote kinase activation of the IRS1, which lies immediately downstream of the IGF-I receptor (Guilherme & Czech 1998, Lebrun et al. 2000). In addition, the IGF-I receptor has been shown to regulate vascular endothelial growth factor-C (VEGF-C) expression, and IGF-I ligand has been shown to induce in vitro expression of VEGF-C, the overexpression of which was also a marker of OCPP-defined poor prognosis in our previous study (Tang et al. 2003). Furthermore, caveolin-1 and plasminogen activator inhibitor-1, also markers of poor prognosis by the OCPP, have been shown to upregulate and be upregulated by IGF-I respectively (Ravid et al. 2005).

The downstream IGF signaling pathway was also significantly differentially expressed between the two OCPP-derived patient groups. Evidence of differential expression was shown not only when the pathway was analyzed as a single entity but also in an individual gene-by-gene analysis for several of
the pathway genes (Table 3 and Fig. 2). The downstream signals following IGF-I receptor activation involve the genes shown in Fig. 2 and Table 3, and lead to mitogenic/anti-apoptotic effects. Specifically, the IGF-I receptor activates the PI3 kinase cascade (through IRS 1) as well as the MAP kinase cascade (Raf, MEK, Erk, through SHC1) ultimately leading to activation of mitogenic/anti-apoptotic transcription factors, such as cFos and cJun (LeRoith et al. 1995, LeRoith & Roberts 2003). It is therefore notable that several of these downstream molecules were overexpressed in poor prognosis tumors in our analysis (Table 3). However, it should be noted that genes included in the IGF signaling pathway are generally activated following tyrosine kinase receptor activation by parallel pathways (such as the PI3 kinase and other pathways), and therefore cannot be considered as reflecting exclusively input provided through the IGF axis.

Taken together, these findings suggest that expression changes of the genes in the IGF axis may be one of the features underlying the survival differences captured by the OCPP and that there may be a biologic link between several of the prognostic markers represented in the OCPP and the genes comprising the IGF axis that needs to be further investigated.

Definitive conclusions on the functional significance of the IGF axis in ovarian cancer cannot be reached solely on the basis of static expression profiles, since expression changes do not necessarily signify receptor activation and pathway signaling. Nevertheless, the concerted overexpression of the ligand (IGF-I), the IGF-I receptor, IGFBP1 (which is known to promote IGF binding to the receptor), and several downstream signaling molecules (Tables 2 and 3) in poor prognosis tumors, and the collective association of the IGF axis genes with survival shown in Fig. 1 support the hypothesis that altered tissue expression of the IGF-I axis may play a role in tumor progression in EOC. This is consistent with previous literature demonstrating a growth-promoting effect of IGF overexpression and signaling in other malignancies (LeRoith et al. 1995, Resnicoff et al. 1995, Krueckl et al. 2004).

This study provides a novel comprehensive insight into the relation of the IGF axis to EOC outcome using primary clinical tumor material and suggests that this axis is overexpressed in a subset of tumors and, acting in a paracrine and/or autocrine way may, influence tumor prognosis. Thus, our study adds to prior literature that analyzed the association between one member of this axis (IGF-II) and survival in EOC (Sayer et al. 2005).

Use of antibodies or small molecule inhibitors to block the IGF-I at the receptor level is being explored with encouraging results in various malignancies. In particular, a possible chemosensitizing effect of IGF blockade has been the focus of recent research efforts (Mitsiades et al. 2004, Min et al. 2005, Gotlieb et al. 2006). Additional work is needed to further define and validate a specific IGF-based prognostic tool in EOC, to elucidate the precise role of each of the axis molecules in tumor progression, and to determine whether the expression patterns observed in our study are indeed a marker of IGF axis activation. Confirmation of our findings by biological studies may provide new therapeutic opportunities, and assessment of the expression patterns of the IGF axis genes at diagnosis may help select patients for studies exploring the effects of IGF-I inhibition in ovarian cancer.

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References


Hosack DA, Dennis G Jr, Sherman BT, Lane HC & Lempicki RA 2003 Identifying biological themes within lists of genes with EASE. *Genome Biology* 4 R70.


LeRoith D, Werner H, Beitner-Johnson D & Roberts CT Jr 1995 Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine Reviews* 16 143–163.


Moschos SJ & Mantzoros CS 2002 The role of the IGF system in cancer: from basic to clinical studies and clinical applications. *Oncology* 63 317–332.


