The Relationship of Insulin-Like Growth Factor-II, Insulin-Like Growth Factor Binding Protein-3, and Estrogen Receptor-α Expression to Disease Progression in Epithelial Ovarian Cancer

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Abstract

Purpose: The insulin-like growth factor (IGF) system plays important roles in cancer; blocking IGF signaling has been shown to have therapeutic effects on tumor growth. Many studies have focused on the effect of IGF-I, but few have addressed IGF-II. To assess the role of IGF-II in cancer, we analyzed IGF-II expression in ovarian cancer and examined its association with disease characteristics and prognosis.

Experimental Design: Included in the study were 215 patients with primary epithelial ovarian cancer. Fresh tumor specimens were collected during surgery, and the patients were followed for a median of 31 months. Total RNA was extracted from the tumor and analyzed for IGF-II, IGF binding protein 3 (IGFBP-3), and estrogen receptor-α expressions using quantitative reverse transcription PCR. Survival analysis was done to examine the associations of IGF-II with disease progression.

Results: IGF-II expression was found to be higher in tumors with poor prognosis; this included tumors with advanced stage, poor differentiation, serous histology, and large residual lesions. Patients with high IGF-II had elevated risk for disease progression and death, although the significance became less evident when the analysis was adjusted for clinical and pathologic variables. IGFBP-3 expression was higher in less aggressive tumors, but was not associated with disease progression. The expression of estrogen receptor-α had no effect on survival.

Conclusion: This study found evidence that IGF-II expression is associated with disease progression, suggesting that IGF-II and IGF signaling are potential targets for ovarian cancer treatment. The study also indicates that IGF-II and IGFBP-3 have limited value in prognosis because of their strong associations with disease stage and tumor grade.

Epithelial ovarian cancer is one of the most lethal gynecologic malignancies; more than half of the patients die from the disease within 5 years of their diagnosis (1). Because 90% of ovarian cancers are believed to arise from surface epithelial cells of the ovary (2), repeated ovulation-associated tissue damage and cyclic stimulation by sex steroid hormones or other factors are suspected to be involved in the development of ovarian cancer (3, 4). Mounting evidence also suggests that insulin-like growth factors (IGF) play important roles in carcinogenesis and tumor progression (5, 6). Currently, blocking IGF signaling is under intense investigation as a potential therapeutic target for cancer treatment (7–9).

Many members of the IGF family, including IGF ligands (IGF-I and IGF-II), specific IGF-binding proteins (IGFBP), and IGF receptors (IGF-IR and IR-A), are expressed in ovarian epithelial cells (5). In a culture study of rabbit ovarian mesothelial cells, overexpression of IGF-IR was found to induce malignant transformation of the cells (10). Insulin receptor isoform A (IR-A) expressed during fetus development has a strong binding affinity for IGF-II; *in vitro* experiments have shown that IR-A is expressed in ovarian tumor cells and that the expression can stimulate ovarian tumor growth (11). A recent clinical study found that IGF-II expression (mRNA) was substantially higher in ovarian cancer than in normal ovarian epithelial cells and that high IGF-II expression was associated with advanced stage and unfavorable prognosis (12). Additionally, microarray data has shown that IGFBP expression is significantly altered in ovarian cancer (13). To further assess the effect of IGF-II on ovarian cancer progression, we conducted a clinical study of primary epithelial ovarian cancers. In this study, we measured IGF-II, IGFBP-3, and estrogen receptor-α (ERα) expressions in 215 ovarian tumor samples using quantitative reverse transcription PCR and examined the association of these genes with the clinical and pathologic features of ovarian cancer and with patient survival.

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Materials and Methods

Patients and tumor samples. Between October 1991 and February 2000, 264 patients underwent surgery for ovarian tumors in the Department of Gynecology, Gynecologic Oncology Unit, at the University of Turin in Italy. Of these patients, 23 were diagnosed with metastatic cancer, 19 with benign tumors (histologic grade, 0), 6 with ovarian cancer of nonepithelial origin, and 1 with endometriosis. The remaining 215 patients diagnosed with primary epithelial ovarian cancer were included in this study. The study was approved by the institution's ethical review committee. For these patients, the average age at surgery was 57.6 years (SD, 11.5; range, 26-82). Follow-up information was available for 205 patients who were followed from surgery to June 2001. The overall follow-up time ranged from 0.6 to 114 months, and the median was 31 months. The median disease progression-free survival was 20.6 months.

Disease stage was determined based on the criteria defined by the International Federation of Gynecologists and Obstetricians (14). Of the 214 patients who had information on disease stage, 53 (24.8%) had stage I, 12 (5.6%) had stage II, 134 (62.6%) had stage III, and 15 (7.0%) had stage IV disease. Tumor histologic grade and type were evaluated according to the WHO criteria (15). Thirty-four patients (15.9%) had well-differentiated grade 1 tumors, 40 (18.7%) had grade 2 tumors, and 140 (65.4%) had poorly differentiated grade 3 tumors. Histologic types included: serous papillary (40.0%, $n = 86$), endometrioid (19.5%, $n = 42$), undifferentiated (17.7%, $n = 38$), mucinous (8.4%, $n = 18$), clear cell (7.4%, $n = 16$), mullerian (6.5%, $n = 14$), and other (0.5%, $n = 1$). For data analysis, histologic types were classified into two groups: serous and nonserous. After initial surgery, most of the patients were treated with platinum-based chemotherapy following a standard protocol, which included platinum and cyclophosphamide before 1997 and carboplatinum and paclitaxel after 1997.

Four categories of treatment response were defined in the study, which included (*a*) complete response when there was resolution of all evidence of disease for at least 1 month, (*b*) partial response when there was a decrease of ≥50% in the product of the diameters (maximum and minimum) of all measurable lesions without the development of new lesions for at least 1 month, (*c*) stable disease if there was a decrease of <50% or an increase of <25% in the product of the diameters of all measurable lesions, and (*d*) progressive disease if there was an increase of ≥25% in the product of the diameters of all measurable lesions or the presence of new lesions. One hundred and forty-seven patients (68.4%) had a complete response, 38 patients (17.7%) had a partial response, 4 patients (1.9%) had no response (stable disease), 14 patients (6.5%) had disease progression, and 12 patients (5.6%) had no information available on their treatment response. When analyzed, patients with partial response, no response, and disease progression were classified as one group and compared with patients who had a complete response.

Total RNA extraction and real-time PCR analysis. Fresh tumor samples were collected during surgery. The specimens were snap-frozen in liquid nitrogen immediately after resection and stored at −80°C until analysis. Tissue samples were examined independently by two pathologists to confirm tumor content; the majority of samples contained 80% to 90% tumor cells. Tumor tissues were pulverized manually in liquid nitrogen, and the tissue powder (~100 mg) was processed to extract total RNA following a standard phenol-chloroform protocol. Total RNA was treated with DNase to remove genomic DNA using a DNA-free kit (Ambion, Inc., Austin, TX). Total RNA concentrations were measured using a spectrophotometer; RNA integrity was evaluated after agarose gel electrophoresis.

One microgram of total RNA from each sample was used for reverse transcription according to the cloned AMV First-Strand cDNA synthesis protocol (Invitrogen, Carlsbad, CA). Real-time PCR primers for IGF-II, IGFBP-3, ERα, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; internal control) were designed with the Primer Expression software (Applied Biosystems, Foster City, CA) using GenBank accession numbers BT007013, X64875, U47678, and NM_002046, respectively. The primer sequences were 5′-ACC GTG CTT CCG GAC AAC T (IGF-II forward), 5′-TGG ACT GCT TCC AGG TGT CA (IGF-II reverse), 5′-GGC CAT GAC TGA GGA AAG GA (IGFBP-3 forward), 5′-CCT GAC TTT GCC AGA CCT TCT T (IGFBP-3 reverse), 5′-TAG AGG GCA TGG TGG AGA TCT T (ERα forward), 5′-CAA ACT CCT CTC CCT GCA GAT T (ERα reverse), 5′-GAA GGT GAA GGT CGG AGT C (GAPDH forward), and 5′-GAA GAT GGT GAT GGG ATT TC (GAPDH reverse). Quantitative reverse transcription PCR was done using the Chromo4 continuous fluorescence detector system (MJ Research, Inc., Waltham, MA). In the PCR reaction (20 μL), 0.1 μg of cDNA template was mixed with 10 μL of DyNamo HS SYBR Green qPCR master mix (MJ Research) and a pair of primers at a final concentration of 200 nmol/L for IGFBP3, ERα, and GAPDH, and of 100 nmol/L for IGF-II. PCR thermal cycling conditions included initial incubation at 50°C for 2 minutes, denaturing at 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Melting curves were generated after each PCR to analyze the size of the PCR product. Each sample was tested in triplicate for each gene, and the mean value of the three reactions was used. If the coefficient of variation exceeded 10%, the mean of the closest two reactions was used.

Statistical analysis. Real-time PCR results were recorded as Ct values (threshold cycle), which are defined as the cycle number at which the fluorescent signal generated by the SYBR green dye and double-stranded DNA complex is statistically higher than the background signal. To adjust for different concentrations of total cDNA in the samples, a ΔCt was calculated based on the difference in Ct values between the target gene and the housekeeping gene GAPDH. ΔCt is inversely proportional to the mRNA level of a given gene. In data analysis, ΔCt was converted into an expression index based on the formula: $1{,}000 \times 2^{(-\Delta Ct)}$. Associations of IGF-II expression with clinical and pathologic variables of ovarian cancer were analyzed using the nonparametric Wilcoxon rank-sum test. Correlations of gene expression with numerical or ordinal variables, such as age at surgery, disease stage or tumor grade, were analyzed using the Spearman correlation coefficient. Survival analysis was done using Cox proportional hazards regression to examine the association of gene expression with risk of disease progression or death. Progression-free survival was defined as the time interval from the date of surgery to the date of disease progression or last follow-up; overall survival was defined as the time interval between the date of surgery and the date of last follow-up or death. In survival analyses, IGF-II expression was analyzed categorically using its tertile distribution; hazards ratios (relative risks) and their 95% confidence intervals were calculated. Analysis was done using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Expression of IGF-II, IGFBP3, and ERα. Sufficient RNA was obtained from 206 patients; of these, 187 had detectable IGF-II mRNA. IGF-II expression ranged widely from 0.01 to 99,479.9 expression index (median, 12.43). For IGFBP-3 and ERα, the number of samples with detectable mRNA were 198 and 190, respectively. The median expressions were 95.96; expression index for IGFBP-3 (range, 2.19-89,366.8) and 65.67 expression index for ERα (range, 0.10-32,740.4). Among the three genes, IGFBP-3 had the highest level of expression, whereas the expression of IGF-II was the lowest. IGF-II expression did not correlate with the expression of IGFBP-3 ($r = 0.13$, $P = 0.083$) or ERα ($r = 0.12$, $P = 0.106$; Table 1), but IGFBP-3 and ERα expressions were positively correlated ($r = 0.31$, $P < 0.001$).

Associations of gene expression with disease characteristics. Table 1 shows that IGF-II expression was positively correlated with disease stage ($r = 0.23$, $P = 0.001$), tumor grade ($r = 0.21$, $P = 0.002$), and residual tumor size ($r = 0.22$, $P = 0.002$),

Table 1. Spearman correlation of IGF-II, IGFBP-3, and ERα expressions with clinicopathologic and survival features

Variable	IGF-II, correlation coefficient (*P*)	IGFBP-3, correlation coefficient (*P*)	ERα, correlation coefficient (*P*)
Age (*n* = 202)	0.11 (0.121)	0.13 (0.058)	0.04 (0.543)
Disease stage (*n* = 205)	0.23 (0.001)	−0.21 (0.003)	−0.01 (0.859)
Tumor grade (*n* = 205)	0.21 (0.002)	−0.11 (0.123)	−0.09 (0.188)
Residual tumor size (*n* = 199)	0.22 (0.002)	−0.18 (0.009)	−0.00 (0.991)
ERα (*n* = 173)	0.12 (0.106)	0.31 (<0.001)	
IGFBP-3 (*n* = 173)	0.13 (0.083)		

whereas IGFBP-3 expression was inversely correlated with disease stage ($r = -0.21$, $P = 0.003$) and residual tumor size ($r = -0.18$, $P = 0.009$). No correlation was found between ERα expression and any of the clinical variables examined. Increases in patient age at surgery did not seem to affect the expression of IGF-II or ERα, but IGFBP-3 expression was slightly elevated with age ($r = 0.13$, $P = 0.058$). Table 2 compares the median expressions of these genes by different clinical and pathologic characteristics. IGF-II expression was significantly higher in advanced (stages III and IV) than in early disease (stages I and II; 27.06 versus 5.48, $P = 0.020$), and in poorly differentiated tumors (grade 3) than in well-differentiated tumors (grades 1 or 2; 24.92 versus 6.85; $P = 0.051$). Patients with either suboptimal debulking or with residual tumor after surgery also had significantly higher IGF-II expression than patients with optimal debulking or no residual tumor ($P = 0.015$ and 0.024, respectively). Patients who had complete response to treatment had lower IGF-II expressions than patients who had partial or no response, although the difference was not statistically significant (11.14 versus 45.24; $P = 0.178$). IGF-II expression

Table 2. Associations of IGF-II, IGFBP-3, and ERα expression with clinical and pathologic characteristics of ovarian cancer

Variable	IGF-II		IGFBP-3		ERα	
	n	Median (5th-95th)*	*n*	Median (5th-95th)*	*n*	Median (5th-95th)*
Disease stage						
I-II	48	5.48 (0.19-2881.19)	57	211.64 (13.24-13670.56)	53	113.83 (0.60-3754.19)
III-IV	138	27.06 (0.15-5660.78)	140	74.95 (6.63-1945.73)	136	69.07 (0.55-727.83)
P†		0.020		<0.001		0.619
Tumor grade						
1-2	57	6.85 (0.11-1795.02)	63	185.87 (11.24-5915.50)	62	98.95 (0.62-1054.82)
3	129	24.92 (0.18-6587.21)	134	86.48 (10.12-2253.76)	127	68.82 (0.55-727.83)
P		0.051		0.020		0.173
Histologic type‡						
Nonserous	75	14.30 (0.18-3008.41)	83	147.80 (12.08-5915.50)	76	87.49 (0.60-1054.82)
Serous	112	19.33 (0.15-6587.21)	115	73.51 (6.59-2964.93)	114	77.55 (0.55-802.64)
P		0.487		0.008		0.404
Debulking result						
Optimal	89	8.78 (0.18-2077.24)	96	135.87 (11.81-10895.55)	91	91.23 (0.62-1041.98)
Suboptimal	93	36.77 (0.15-6587.21)	96	89.05 (6.67-2861.95)	95	63.15 (0.54-999.08)
P		0.015		0.040		0.683
Residual tumor size (cm)						
0	74	8.92 (0.18-2077.24)	83	149.93 (16.73-10895.55)	77	97.33 (0.60-1184.55)
>0	107	31.47 (0.15-6180.26)	108	74.95 (6.590-2253.76)	108	62.06 (0.55-727.83)
P		0.024		0.003		0.524
Response to treatment§						
Yes	124	11.14 (0.19-3008.41)	134	116.32 (10.14-5915.50)	130	91.20 (0.62-1054.82)
No	52	45.24 (0.05-5660.78)	52	556.63(5.94-2861.95)	52	56.77 (0.54-492.60)
P		0.178		0.070		0.185

*Expression levels at the 5th and 95th percentiles.
†Wilcoxon two-sample test with t approximation.
‡Nonserous tumors include clear cells, endometrioid, mucinous, mullarian, and undifferentiated.
§No response to treatment includes no response, partial response, and progressive disease.

varied by histologic types and was higher in serous than in nonserous tumors; however, this difference was not statistically significant ($P = 0.085$).

Associations between IGFBP-3 expression and disease characteristics were in the opposite direction compared with IGF-II (Table 2). Patients with high IGFBP-3 expression were more likely to be those with early stage disease ($P < 0.001$), well differentiated tumors ($P = 0.020$), optimal debulking ($P = 0.040$), and no residual tumor postsurgery ($P = 0.003$). Although IGFBP-3 was also higher in patients with complete response to treatment than in those with partial or no response ($P = 0.070$), as well as in nonserous than in serous tumors ($P = 0.140$), these differences were not statistically significant. The expression of ERα was not associated with any of the variables (Table 2).

Expression data was also analyzed categorically by grouping the expression level into three categories, low, medium and high, based on tertile distributions. Patients with undetectable expressions were included in the low-expression category. Similar to the findings in Table 2, IGF-II expression was associated with disease stage, tumor grade, residual tumor size, debulking results, and was significantly higher in serous than in nonserous tumors (Fig. 1). IGFBP-3 expression was again associated with disease stage, residual tumor size, and debulking results in a direction opposite to that of IGF-II (Fig. 1). No association with ERα expression was found for these clinicopathologic variables with the exception of histologic type. ERα expression was higher in serous than in nonserous tumors (Fig. 1).

Associations of gene expression with patient survival. High IGF-II expression was significantly associated with increased risk of disease progression ($P = 0.016$) and death ($P = 0.005$) in unadjusted analysis (Table 3). Compared to those with low IGF-II expression, patients with high expression had a 1.87-fold increase in risk of disease progression and a 2.11-fold increase in risk of death. These associations remained significant after the regression models were adjusted for IGFBP-3 and ERα expression ($P = 0.014$ for both). However, when the Cox models were adjusted for disease stage, tumor grade, and histologic type, the association with disease progression disappeared ($P = 0.271$), and the association with death was reduced to borderline significance ($P = 0.072$). IGFBP-3 (Table 3) and ERα expression (data not shown) was not associated with disease progression or death.

Discussion

In this clinical study, we found that IGF-II and IGFBP-3 expressions in ovarian cancer were associated with several prognostic indicators. Patients with advanced stage disease, poorly differentiated tumor, or residual tumor after surgery tended to have higher IGF-II and lower IGFBP-3 expressions compared to those with early stage, low grade, or complete tumor removal. This study also found that IGF-II expression was associated with patient survival; patients with high levels of IGF-II mRNA were more likely to have disease progression or to die than those with low IGF-II. The significance of these associations was reduced after the analysis was adjusted for disease stage, tumor grade, and histologic type; given the strong link between IGF-II expression and these clinical and pathologic variables, this change suggests that the effects of IGF-II on survival are appreciably mediated through tumors with more deleterious properties.

The tissue specimens used in this study were fresh-frozen tumor samples collected by the pathologists who evaluated the

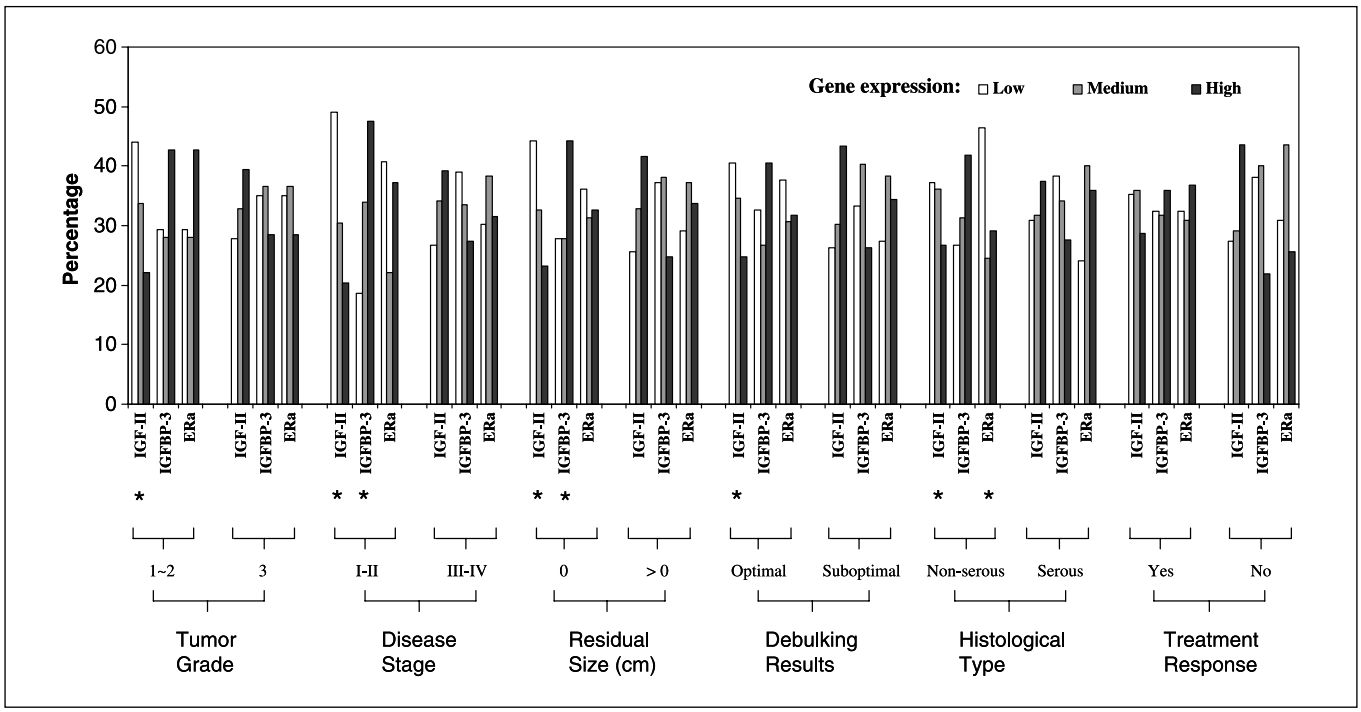

Fig. 1. IGF-II, IGFBP-3, and ERα expressions were classified into low, medium, and high categories based on their tertile distributions. The percentages of IGF-II, IGFBP-3, and ERα expressions in each category were compared between patients with different tumor grade, disease stage, residual tumor size, debulking results, histologic type, and response to treatment. *, $P < 0.05$, significant association.

Table 3. Associations of IGF-II and IGFBP-3 expression with ovarian cancer progression and death

	Progression-free survival	Overall survival
	HR* (95% CI)	HR* (95% CI)
IGF-II expression		
Unadjusted analysis		
Low	1.00	1.00
Medium	1.41 (0.84-2.37)	1.63 (0.96-2.75)
High	1.87 (1.12-3.10)	2.11 (1.25-3.55)
Test for trend	$P = 0.016$	$P = 0.005$
Adjusted analysis†		
Low	1.00	1.00
Medium	1.33 (0.79-2.27)	1.45 (0.85-2.48)
High	1.76 (1.05-2.94)	1.94 (1.14-3.29)
Test for trend	$P = 0.014$	$P = 0.014$
Adjusted analysis‡		
Low	1.00	1.00
Medium	1.39 (0.83-2.34)	1.45 (0.85-2.47)
High	1.33 (0.80-2.23)	1.62 (0.96-2.72)
Test for trend	$P = 0.271$	$P = 0.072$
IGFBP-3 expression		
Unadjusted analysis		
Low	1.00	1.00
Medium	1.49 (0.92-2.40)	1.42 (0.89-2.29)
High	0.80 (0.47-1.36)	0.77 (0.45-1.32)
Test for trend	$P = 0.473$	$P = 0.432$
Adjusted analysis†		
Low	1.00	1.00
Medium	1.37 (0.84-2.26)	1.32 (0.81-2.14)
High	0.79 (0.45-1.37)	0.83 (0.47-1.47)
Test for trend	$P = 0.404$	$P = 0.631$
Adjusted analysis‡		
Low	1.00	1.00
Medium	1.65 (1.02-2.68)	1.63 (1.00-2.64)
High	1.03 (0.60-1.75)	0.91 (0.53-1.57)
Test for trend	$P = 0.768$	$P = 0.926$

Abbreviation: CI, confidence interval.
*Hazard ratio from Cox proportional hazards regression analysis.
†Adjusted for age, IGFBP-3, and ERα (in IGF-II) or for age and IGF-II (in IGFBP-3).
‡ Adjusted for age, disease stage, tumor grade, and histologic type.

specimens to determine tumor cell content and composition. Tissue sections containing a majority (90%) of tumor cells were dissected from the frozen samples during the assessment and stored for the study. Although we cannot rule out the possibility that some samples may contain normal cells or stromal tissue, the extent of nontumor tissue contamination is likely to be minimal due to the method of collection. Thus, bias due to tissue selection is unlikely to affect the results of our study.

The findings of our study are generally in agreement with the observations of other studies. Sayer et al. reported recently that IGF-II expression was 300-fold higher in ovarian cancer than normal tissues, and that high expression was associated with advanced stage and poor prognosis (12). High IGF-II expression has been seen, not only in ovarian cancer, but also in other cancers. IGF-II expression was increased in prostate cancer when compared with nonmalignant prostate tissues (16, 17), and the increased expression was associated with prostate cancer progression (18). High levels of IGF-II expression were also found in breast cancer (19, 20), gastric cancer (21, 22), and esophageal cancer (23). In colorectal cancer, both IGF-II mRNA and protein were shown to be elevated compared with normal tissue, and the elevation was associated with advanced disease stage and poor prognosis (24–26).

Although many studies have shown increased expression of IGF-II in tumor tissues, the molecular mechanism explaining the change in expression remains largely unknown. Under normal circumstances, IGF-II transcription is subject to imprinting, and loss of imprinting (LOI) can lead to the overexpression of IGF-II, which has been found frequently in cancer (27, 28). A recent study by Cui et al. showed that LOI existed in 30% of colon cancers compared with only 10% of normal colon samples, and that individuals with LOI in IGF-II had a >20-fold increase in risk of colorectal cancer (29). These investigators also developed a transgenic model to show that LOI in IGF-II causes increases in IGF-II transcription and translation and that the risk of developing intestinal tumors is elevated considerably in the LOI animals compared with the controls (30). We were unable to determine whether high IGF-II expression in ovarian cancer was also due to LOI in our study. However, findings from other studies do not seem to support the link between LOI and elevated IGF-II expression in ovarian cancer. Yun et al. analyzed 27 ovarian tumor samples to examine whether increased IGF-II expression was related to the loss of IGF-II imprinting. Of the tumor specimens evaluated, 11 were informative and all of the 11 samples showed monoallelic expression of IGF-II. The authors concluded that increased expression of IGF-II in ovarian cancer could be due to a mechanism other than LOI (31). A different study found that 6 of 11 informative ovarian tumors had biallelic IGF-II expression, but the samples studied included benign adenomas and cysts (32). Another study evaluated 43 ovarian tumors and detected LOI in only 5 of 20 informative samples (33). Thus, it has been suggested that LOI may not be common in ovarian cancer.

IGF-II has been detected more frequently in local tissues than IGF-I (34–40). This suggests that IGF-II could play a more important role than IGF-I in tumorigenesis and cancer progression. Recent studies show that IGF-II exerts its action not only by binding to the type I IGF receptor (IGF-IR), but also by interacting with a fetal form of the insulin receptor (IR-A). IR-A was found to be overexpressed in a number of malignancies, including ovarian cancer, and cancer cells which predominantly expressed IR-A seemed to have IGF-II overexpression as well (11, 41–43). Given the fact that IGF-II is able to bind to both IGF-IR and IR-A, and that both receptors are activated upon the IGF-II binding to initiate mitogenic and antiapoptotic signals, it is plausible that the effect of IGF-II is substantially amplified in local tissues in which both receptors are present, and meanwhile, overexpression of IGF-II exists. These elements are suspected to form a strong autocrine or paracrine growth-stimulatory loop that promotes cancer progression (44). *In vitro* experiments also suggest that IGF-II may act through IR-A to stimulate cell migration and invasion (42).

IGFBP-3 is a specific IGF binding protein that not only regulates the IGF activity but also exerts IGF-independent

actions that suppress cell proliferation and stimulate apoptosis (45). Although no effect was found on patient survival in our study, high IGFBP-3 expression was associated with some favorable prognostic indicators of the disease including early stage and complete tumor removal during surgery. These findings are consistent with our understanding of the role of IGFBP-3 in cancer, as well as with the results from our previous study examining IGFBP-3 protein levels in ovarian cancer. In our earlier study, we found that levels of IGFBP-3 protein in tumor tissues were inversely associated with disease stage and residual tumor size, but the protein level was not independently associated with disease progression or overall survival (46). Because the associations between IGFBP-3 and disease characteristics were in a direction opposite to those of IGF-II, and because IGFBP-3 may suppress the action of IGF-II, we controlled for IGFBP-3 expression when assessing the effect of IGF-II. The association of IGF-II with both progression-free and overall survival was sustained after adjusting for IGFBP-3; this suggests that the effect of IGF-II on survival outcomes is independent of the effect of IGFBP-3.

The expression of ERα in ovarian cancer was also evaluated in this study. ERα expression was positively correlated with the expression of IGFBP-3, but not with IGF-II. Overall, ERα expression did not vary significantly by disease stage, tumor grade, residual tumor size, or debulking results, although it seemed that less aggressive tumors had slightly higher expression of ERα. Results from other studies have shown inconsistent findings regarding ERα expression in ovarian cancer. Brandenberger et al. (47) found that ERα mRNA either did not change, or else slightly increased in ovarian cancer, whereas Lau et al. (48) reported that ERα expression was significantly lower in ovarian cancer than normal tissue. This inconsistency could relate to tumor histology because in our study, ERα expression was significantly higher in serous than in nonserous tumors. This difference was also observed by Lee et al., who found that 85% of serous tumors had ERα expression compared with 54% of nonserous tumors in a group of 269 ovarian tumor samples (49). Fujimura et al. also reported that ERα expression was absent in clear cell tumors, but was present in serous, endometrioid, and mucinous cells of ovarian cancer (50). A similar finding was also seen in our study; ERα mRNA was substantially lower in clear cell tumors than in other types.

Thus far, no epidemiologic studies have been published assessing circulating IGF-II in relation to ovarian cancer risk. A clinical study reported recently by Mor et al. found that serum levels of IGF-II, determined by antibody microarray and ELISA, were significantly lower in ovarian cancer patients than in control subjects, and that using this peptide hormone in combination with other molecular markers in serum may assist ovarian cancer diagnosis (51). Studies of circulating IGF-II in other cancers have not found convincing evidence in linking IGF-II to cancer risk (52–56). Unlike IGF-I, IGF-II in the circulation is not regulated by growth hormones and does not change with age. Therefore, the effect of IGF-II regulated through the endocrine system is not considered to be as important as that of paracrine or autocrine regulation in local tissue. Clarifying the local effect of IGF-II on cancer progression will help not only to better understand the role of IGF-II in cancer, but also to facilitate the development of therapeutic strategies that target IGF signaling in cancer. Several humanized antibodies against IGF type I receptor (IGF-IR) have been developed and have shown therapeutic effects in animal models (57, 58). Agents other than antibodies are also under intensive investigation and evaluation (7). If the effect of IGF-II on cancer progression is confirmed, then one has to consider that IGF-IR-based treatment strategy may have limitations because IGF-II also interacts with insulin receptor (IR-A). To completely shut down IGF signaling, it may be necessary to consider blocking all three IGF-related receptors (59).

In conclusion, this study shows that IGF-II, IGFBP-3, and ERα mRNA are detectable in most ovarian tumor tissues. The expressions of IGF-II and IGFBP-3 vary substantially by clinical and pathologic features of the disease. High levels of IGF-II expression are associated with unfavorable prognostic indicators of the disease, whereas high expression of IGFBP-3 is related to favorable prognostic variables. Furthermore, the expression of IGF-II is associated with both progression-free survival and overall survival; patients with high IGF-II expression tend to have higher risk of disease progression and death regardless of the level of IGFBP-3 expression. However, the strength of the association is reduced after adjusting for clinicopathologic variables. Although IGF-II does not seem to be an independent marker for ovarian cancer prognosis, the findings of this study suggest that postoperative treatment suppressing IGF signaling may help slow or prevent disease progression.

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