Research Article

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Endometrial Cancer Insulin-Like Growth Factor 1 Receptor (IGF1R) Expression Increases with Body Mass Index and Is Associated with Pathologic Extent and Prognosis

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Abstract

Background: Obesity is a main risk factor for endometrial carcinoma (EC). Insulin-like growth factor 1 receptor (IGF1R) expression may influence this association.

Methods: IGF1R IHC was performed on a tissue microarray with 894 EC and scored according to the percentage and intensity of staining to create immunoreactivity scores, which were dichotomized into low and high IGF1R expression groups. Logistic regression modeling assessed associations with body mass index (BMI), age, histology, pathologic extent of disease (pT), and lymph node metastasis (pN). Overall survival (OS) and disease-free survival (DFS) were compared between IGF1R expression groups using Kaplan–Meier curves and log-rank tests.

Results: The proportion of patients with high IGF1R expression increased as BMI (<30, 30–39, and 40+ kg/m^2) increased (P = 0.002). The adjusted odds of having high IGF1R expression was 1.49 [95% confidence interval (CI), 1.05–2.10, P = 0.024] for patients with BMI 30 to 39 kg/m^2 compared with <30 kg/m^2 and 1.62 (95% CI, 1.13–2.33, P = 0.009) for patients with BMI 40+ kg/m^2 compared with <30 kg/m^2. High IGF1R expression was associated with pT and pN univariately and with pT after adjusting for BMI, pN, age, and histologic subtype. DFS and OS were better with high IGF1R expression, P = 0.020 and P = 0.002, respectively, but DFS was not significant after adjusting for pT, pN, and histologic subtype of the tumor.

Conclusions: There is an association between BMI and EC IGF1R expression. Higher IGF1R expression is associated with lower pT and better DFS and OS.

Impact: These findings suggest a link between IGF1R EC expression and obesity, as well as IGF1R expression and survival. *Cancer Epidemiol Biomarkers Prev; 25(3); 438–45. ©2015 AACR.*

Introduction

Endometrial carcinoma (EC) is the most prevalent gynecologic cancer in the United States. Obesity, which affects approximately 35% of the U.S. population (1), is the main risk factor for EC (2–4). This association has been attributed to increased and unopposed estrogen bioavailability (5). However, other attendant metabolic and hormonal abnormalities may strongly influence the endometrial environment. Abnormalities in the IGF-1 axis are prevalent in obese patients (6), and crosstalk between estrogen and insulin– and insulin-like growth factor (IGF)–related pathways (7–9) may have a pathophysiologic role.

EC cell line studies have confirmed increased proliferation when exposed to IGF-1 and estrogen, increased autocrine production of IGF-1 when exposed to estrogen, and decreased proliferation under IGF-1 in the presence of IGF-1–binding proteins (8, 10–12). However, conflicting serum studies have reported null, positive, and negative associations between cancer risk and levels of insulin, C-peptide, IGF-1, and binding proteins (13–20).

Analysis of IGF1R in EC has been reported for a limited number of samples with mixed results (21–26). We analyzed tumor expression of IGF1R in 894 EC hysterectomy specimens and correlated expression with clinicopathologic factors [age, body mass index (BMI), histologic subtype, pT, and pN], as well as with recurrence and survival.

Materials and Methods

The Ohio State University pathology database was searched for hysterectomy specimens with endometrial carcinoma from April 2007 to 2012. Clinical and pathologic data, including BMI, age, pathologic extent of the primary tumor ("pT," according to the American Joint Committee on Cancer Staging Handbook, seventh edition; ref. 27), lymph node status, histologic subtype, histologic grade, and follow-up data, were extracted from electronic medical records and pathology reports. A tissue microarray (TMA) with 1,909 cores representing 896 consecutive hysterectomy specimens with EC was constructed. Each tumor was represented with single to triplicate TMA cores (1 core, n = 393; 2 cores, n = 18; 3 cores, n = 483).

Sections of each paraffin-embedded TMA block were cut at 4 μm and placed on positively charged slides. Slides were placed in a 60°C oven for 1 hour and cooled before placement on the Leica

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BondMax Autostainer. All slides were deparaffinized and rehydrated with Bond Dewax Solution (product code AR9222) and 100% alcohol.

All slides were stained with the BOND Protocol IHC-F1 using IGF1R (rabbit mAb G11, catalog number 790-4346; Ventana). Slides were quenched for 5 minutes in a 3% hydrogen peroxide solution to block for endogenous peroxidase. Antigen retrieval was performed using Leica's Bond Epitope Retrieval Solution 1 (ER1, product code AR9961) for 30 minutes. Primary antibodies were incubated for 15 minutes at room temperature. The detection system used for all antibodies was Leica's Bond Polymer Refine Detection (product code DS9800). Finally, sections were incubated with DAB mixed on-line for 10 minutes. Slides were then counterstained in Richard Allen hematoxylin, dehydrated through graded ethanol solutions, and coverslipped.

EC cores were scored blindly by a single pathologist according to the percentage of positive staining (0%–5% = 0, 6%–25% = 1, 26%–50% = 2, ≥51% = 3) and staining intensity (0–3+, corresponding to negative, weak, moderate and strong intensities, respectively). Immunoreactivity scores (IRS) were obtained by multiplying these two values, as has been previously reported for IGF1R (28). Possible IRS included values of 0, 1, 2, 3, 4, 6 and 9. IRS was dichotomized into low and high expression groups (IRS scores of 0–3 and 4–9, respectively). Logistic regression was used to assess the association of BMI and IGF1R expression univariately and while adjusting for age, histologic subtype, pathologic extent of disease (pT), and lymph node metastasis (pN) in a multivariable model. Patients with BMI values of <30 kg/m^2 were used as the reference group. In cases where two to three cores from a single case were present, the core with the highest IRS score was used to decide expression group classification. Overall survival (OS) and disease-free survival (DFS) were compared between IGF1R expression groups using Kaplan–Meier curves and log-rank tests. OS was defined as time from surgery date to death from any cause. Patients were censored if alive (with or without disease) at the time of last follow-up, or if no clear outcome data were available ($n = 3$). DFS was defined as time from the date of surgery to first recurrence. For the DFS analysis, patients were censored if they were alive without disease at the time of last follow-up, were disease free and died of other causes not directly related to the EC (including perioperative deaths), or no clear outcome data were available ($n = 6$). Multivariable Cox proportional hazard models were used to estimate survival HRs according to IRS. We used a stepwise modeling procedure starting with the IGF1R expression group in the model and all significant univariate predictors at the 0.1 level. Predictors with the highest P values were systematically removed from the model until the final model with all significant P values remained. All statistical analyses were performed using Stata 13 (Statacorp LP) or SAS 9.3 (SAS Institute Inc.).

Whole sections of selected tumors represented by a single TMA core were stained with IGF1R IHC by the same procedure outlined above. These included predominantly negative or low IGF1R expression tumors ($n = 31$), and representative high IGF1R expression tumors ($n = 18$). Whole sections were given a separate IRS, with the pathologist blinded to clinicopathologic data and to the previously assigned TMA core IRS data. Weighted kappa statistic and Spearman correlation coefficient were calculated to compare TMA core versus whole-section IRS.

Results

Summary statistics

Of note, 1,882 cores representing 894 cases were included in the study (Table 1) after 27 of the original TMA cores were excluded due to no definite tumor being present on the IGF1R-stained section. Consequently, two cases (both FIGO grade 1 endometrioid carcinomas with single TMA cores) were excluded. Cases represented in the TMA included 750 (83.9%) endometrioid adenocarcinomas [513 (68.4%) FIGO 1, 173 (23.1%) FIGO 2, 64 (8.5%) FIGO 3], 41 (4.6%) serous carcinomas, 10 (1.1%) clear cell carcinomas, 30 (3.4%) carcinosarcomas, 59 (7.9%) carcinomas with mixed epithelial histology, and 4 (0.4%) dedifferentiated or undifferentiated carcinomas. Histologic subtypes were combined into six groups for analysis: FIGO 1 endometrioid, FIGO 2 endometrioid, FIGO 3 endometrioid, serous, carcinosarcoma, and "other" (including clear cell carcinoma, mixed epithelial carcinoma, and dedifferentiated/undifferentiated carcinomas).

Median patient age was 61 (range, 26–93) years. Median BMI was 35.3 (range, 14.7–85.0) kg/m^2. The majority of cases ($n = 563$, 63.0%) presented with pT1a disease, whereas 161 (18.0%), 72 (8.1%), and 99 (11.1%) patients presented with pT1b, pT2, and pT3 or pT4 disease, respectively. Lymph node dissections were performed in 752 cases (84.1%) with 659 (87.6%) of these patients demonstrating N0 status (no regional lymph node metastases), 49 (6.5%) patients demonstrating N1 status (metastasis to pelvic lymph nodes), and 44 (5.9%) patients demonstrating N2 status (metastasis to para-aortic lymph nodes, with or without positive pelvic lymph nodes).

IGF1R IHC

IGF1R pattern of IHC staining was patchy, membranous, and cytoplasmic, with residual benign endometrial glands often serving as an intensely positive internal control (Fig. 1). Sixty-one percent of the tumors had IRS from 4 to 9, placing them in the high IGF1R expression group, and 39% had scores of 0 to 3, placing them in the low IGF1R expression group. Nineteen (38.8%) of the cases with single TMA cores for which subsequent whole-section IHC was performed demonstrated agreement between the two assigned IRS scores; the remaining 30 cases (61.2%) showed predominantly slightly increased IRS values in the whole sections compared with the TMA cores. The Spearman's correlation coefficient was 0.757 ($P < 0.001$), showing a strong association of IGF1R expression between TMA cores and whole sections. The weighted kappa statistic was 0.44 [95% confidence interval (CI), 0.29–0.59], showing moderate correlation between these two groups.

IGF1R clinicopathologic associations

Overall there was a significant difference in IGF1R expression group distribution between BMI categories (<30, ≥30–<40, and ≥40 kg/m^2), our primary interest in this study, with the proportion of patients with high expression increasing as BMI increased ($P = 0.002$). The unadjusted odds of having high IGF1R expression was 1.51 (95% CI, 1.08–2.11, $P = 0.015$) for patients with BMI ≥30 to <40 kg/m^2 compared with those with BMI <30 kg/m^2 (reference group) and 1.83 (95% CI, 1.30–2.57, $P = 0.001$) for patients with BMI ≥40 kg/m^2 compared with those with BMI of <30 kg/m^2. The ORs remained significant (OR, 1.49; 95% CI,

Table 1. Summary statistics of the EC cohort

	Low IGF1R expression	High IGF1R expression	Median BMI, kg/m^2 (range)	BMI <30	30 ≤ BMI <40	BMI ≥40	pT1a	pT1b	pT2	pT3 or pT4
BMI <30 (n = 256, 28.6%)	122 (47.7%)	134 (52.3%)	—	—	—	—	139 (54.3%)	53 (20.7%)	32 (12.5%)	32 (12.5%)
BMI ≥30 to BMI <40 (n = 322, 36.0%)	121 (37.6%)	201 (62.4%)	—	—	—	—	189 (58.7%)	66 (20.5%)	22 (6.8%)	45 (14.0%)
BMI >40 (n = 316, 35.4%)	105 (33.2%)	211 (66.8%)	—	—	—	—	235 (74.4%)	41 (13.0%)	18 (5.7%)	22 (7.0%)
Age, median (range)	62 (33–93)	61 (26–91)	—	65 (34–93)	62 (26–91)	59 (30–83)	60 (26–92)	65 (41–93)	65 (41–91)	62 (36–89)
Endometrioid carcinoma (n = 750, 83.9%)	288 (38.4%)	462 (61.6%)	36.4 (14.7–85.0)	194 (25.9%)	259 (34.5%)	297 (39.6%)	517 (68.9%)	126 (16.8%)	46 (6.1%)	61 (8.1%)
FIGO 1 (n = 513, 68.4%)	173 (33.7%)	340 (66.3%)	37.5 (14.7–74.6)	116 (22.6%)	176 (34.3%)	221 (43.1%)	400 (78.0%)	73 (14.2%)	22 (4.3%)	18 (3.5%)
FIGO 2 (n = 173, 23.1%)	83 (48.0%)	90 (52.0%)	34.9 (16.5–85.0)	53 (30.6%)	60 (34.7%)	60 (34.7%)	92 (53.2%)	39 (22.5%)	17 (9.8%)	25 (14.5%)
FIGO 3 (n = 64, 8.5%)	32 (50.0%)	32 (50.0%)	32.5 (18.0–64.0)	26 (40.6%)	21 (32.8%)	17 (26.6%)	25 (39.1%)	14 (21.9%)	7 (10.9%)	18 (28.1%)
Serous carcinoma (n = 41, 4.6%)	12 (29.3%)	29 (70.7%)	29.5 (18.0–48.4)	22 (53.7%)	16 (39.0%)	3 (7.3%)	14 (34.1%)	5 (12.2%)	7 (17.1%)	15 (36.6%)
Carcinosarcoma (n = 30, 3.4%)	10 (33.3%)	20 (66.7%)	33.0 (23.6–50.8)	9 (30%)	18 (60%)	3 (10%)	5 (16.7%)	7 (23.3%)	9 (30.0%)	9 (30.0%)
Clear cell carcinoma (n = 10, 1.1%)[a]	6 (60.0%)	4 (40.0%)	30.6 (19.5–39.4)	5 (50%)	5 (50%)	0 (0.0%)	5 (50.0%)	2 (20.0%)	2 (20.0%)	1 (10.0%)
Dedifferentiated/undifferentiated carcinoma (n = 4, 0.4%)[a]	3 (75.0%)	1 (25.0%)	28.4 (22.5–39.9)	2 (50%)	2 (50%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	0 (0.0%)	2 (50.0%)
Mixed epithelial carcinoma (n = 59, 6.6%)[a]	29 (49.2%)	30 (50.8%)	32.1 (18.7–82.4)	23 (39.0%)	24 (40.7%)	12 (20.3%)	22 (37.3%)	18 (30.5%)	8 (13.6%)	11 (18.6%)
pT1a (n = 563, 63.0%)	180 (32.0%)	383 (68.0%)	37.2 (17.8–85.0)	139 (24.7%)	189 (33.6%)	235 (41.7%)	—	—	—	—
pT1b (n = 160, 17.9%)	83 (51.9%)	77 (48.1%)	33.3 (14.7–82.4)	53 (33.1%)	66 (41.2%)	41 (25.6%)	—	—	—	—
pT2 (n = 72, 8.1%)	34 (47.2%)	38 (52.8%)	32.0 (19.5–73.0)	32 (44.4%)	22 (30.6%)	18 (25.0%)	—	—	—	—
pT3 or pT4 (n = 99, 11.1%)	51 (51.5%)	48 (48.5%)	33.5 (18.0–59.0)	32 (32.3%)	45 (45.5%)	22 (22.2%)	—	—	—	—
pN0 (n = 659, 73.7%)	250 (37.9%)	409 (62.1%)	34.9 (14.7–74.6)	195 (29.6%)	243 (36.9%)	221 (33.5%)	452 (68.6%)	129 (19.6%)	46 (7.0%)	32 (4.9%)
pN1 (n = 49, 5.5%)	26 (53.1%)	23 (46.9%)	32.2 (20.1–54.2)	18 (36.7%)	20 (40.8%)	11 (22.4%)	7 (14.3%)	11 (22.4%)	8 (16.3%)	23 (46.9%)
pN2 (n = 44, 4.9%)	24 (54.5%)	20 (45.5%)	31.2 (19.5–48.1)	18 (40.9%)	20 (45.5%)	6 (13.6%)	1 (2.3%)	7 (15.9%)	7 (15.9%)	29 (65.9%)
pNX (n = 142, 15.9%)	48 (33.8%)	94 (66.2%)	43.0 (17.8–85.0)	25 (17.6%)	39 (27.5%)	78 (54.9%)	103 (72.5%)	13 (9.2%)	11 (7.7%)	15 (10.6%)

NOTE: Absolute numbers and percentages by row are presented unless otherwise specified by the column or row title.

[a]Clear cell carcinoma, dedifferentiated/undifferentiated carcinoma, and mixed epithelial carcinoma were later redefined as "other" for statistical analysis (see text).

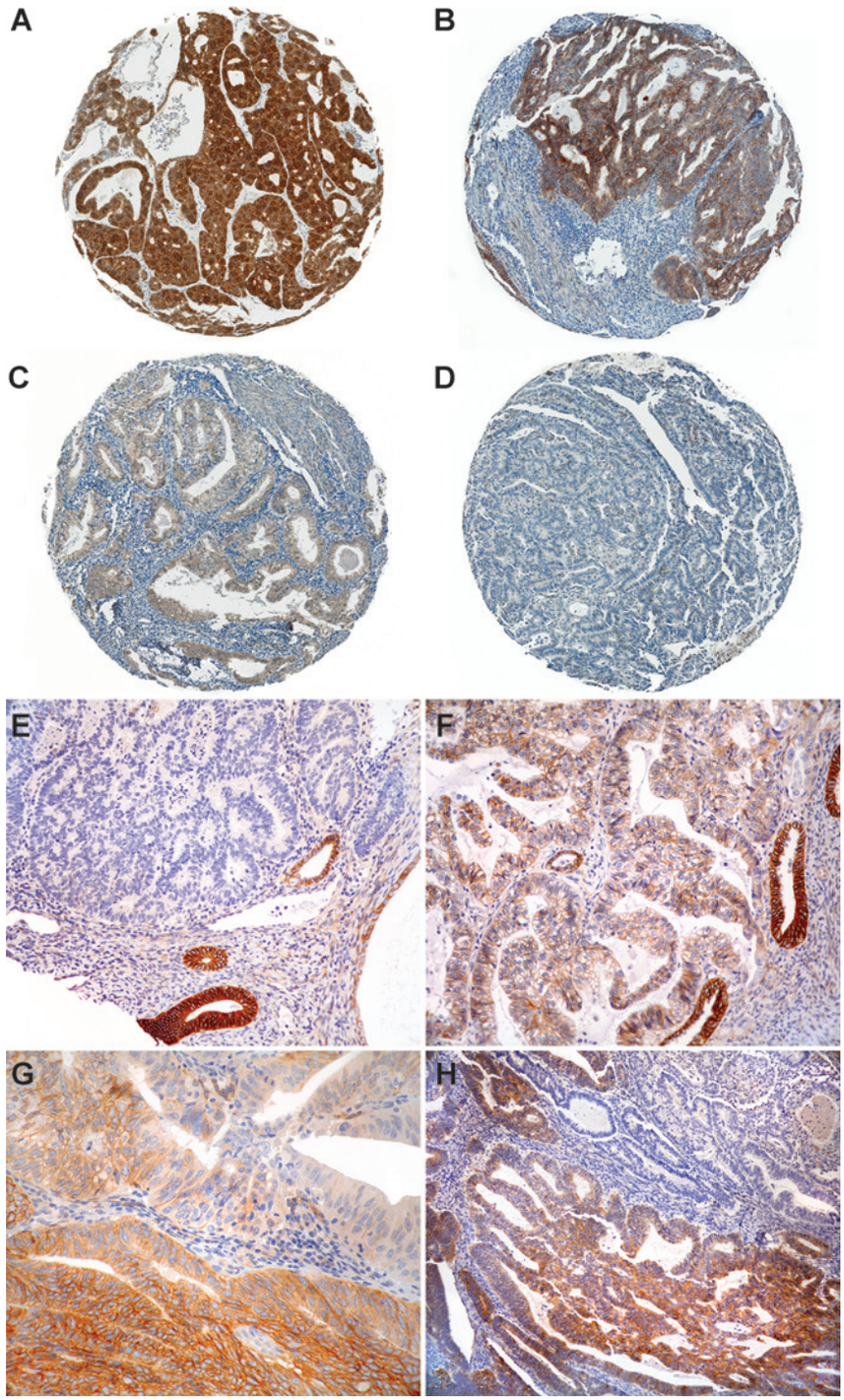

Figure 1.
IGF1R IHC patterns. TMA cores with corresponding intensities of 3+ (A), 2+ (B), 1+ (C), and 0 (D) are demonstrated in four TMA cores. Note that staining patterns are predominantly membranous with some background cytoplasmic staining. Strongly staining benign endometrial glands were often demonstrated adjacent to adenocarcinoma with decreased or absent staining (E and F). Adjacent tumor areas with different IGF1R IHC staining intensities (G and H) demonstrate tumor heterogeneity. In scoring whole sections with the heterogenous or variable staining pattern, the strongest staining intensity was used.

1.05–2.10 and OR, 1.62; 95% CI, 1.13–2.33, respectively) after adjusting for pT, pN, age, and histologic subtype (Fig. 2, Table 2).

Among the other variables explored, univariate results for pathologic extent of the primary tumor demonstrated significantly lower odds of having high IGF1R expression for pT1b (OR, 0.44; 95% CI, 0.31–0.62; $P < 0.001$), pT2 (OR, 0.53; 95% CI, 0.32–0.86; $P = 0.011$), and pT3 or pT4 (OR, 0.44; 95% CI, 0.29–0.68; $P < 0.001$), compared with pT1a. The ORs remained significant after adjusting for BMI, pN, age, and histologic subtype (OR, 0.49; 95% CI, 0.34–0.72; $P < 0.001$; OR, 0.58; 95% CI, 0.34–1.00; $P = 0.048$; OR, 0.51; 95% CI, 0.30–0.88; $P = 0.015$, respectively).

Univariately, FIGO grade 2 endometrioid and FIGO grade 3 endometrioid tumors had lower odds of high IGF1R expression (OR, 0.55; 95% CI, 0.39–0.78; $P < 0.001$ and OR, 0.51; 95% CI,

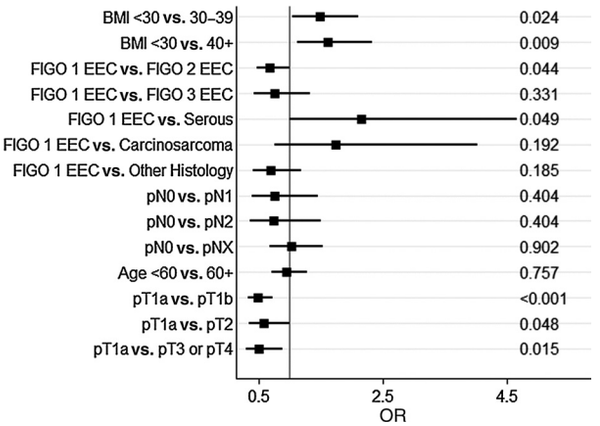

Figure 2.
Multivariable model forest plot, demonstrating ORs, 95% CIs, and *P* values of having high IGF1R expression for various clinical and pathologic variables (EEC, endometrioid endometrial carcinoma).

0.30–0.89; $P = 0.011$, respectively) when compared with the FIGO 1 endometrioid reference group. Serous histology and carcinosarcoma histology had slightly higher odds of high IGF1R expression but were not significant (OR, 1.23; 95% CI, 0.61–2.47; $P = 0.561$ and OR, 1.02; 95% CI, 0.47–2.22; $P = 0.965$, respectively) when compared with the FIGO 1 endometrioid reference group. After adjusting for BMI, age, pT, and pN, there were still lower odds of high IGF1R expression for FIGO grade 2 and 3 endometrioid tumors compared with FIGO grade 1 endometrioid tumors (OR, 0.69; 95% CI, 0.48–0.99; $P = 0.044$ and 0.76; 95% CI, 0.43–1.33; $P = 0.331$, respectively). Serous and carcinosarcoma histologies showed higher odds of high IGF1R expression compared with grade 1 endometrioid tumors after adjusting for BMI, age, pT, and pN (OR, 2.16; 95% CI, 1.00–4.66; $P = 0.049$ and OR, 1.74; 95% CI, 0.76–4.03; $P = 0.185$, respectively).

Patients with pN1 and pN2 had lower odds of having high IGF1R expression when compared with pN0 patients (OR, 0.54; 95% CI, 0.30–0.97; $P = 0.039$ and OR, 0.51; 95% CI, 0.28–0.94; $P = 0.031$, respectively). The ORs were not significant after adjusting for BMI, age, and pT (OR, 0.76; 95% CI, 0.40–1.45; $P = 0.404$ and OR, 0.74; 95% CI, 0.36–1.51; $P = 0.404$ for pN1 and pN2 compared with pN0, respectively). Comparison of pNX with pN0 patients was not significant, OR, 1.03; 95% CI, 0.69–1.53; $P = 0.902$.

Finally, age did not demonstrate an association with IGF1R expression in either univariate or multivariable models.

Table 2. Multivariable logistic regression illustrating ORs of having high IGF1R expression for the various clinicopathologic factor groups

Clinicopathologic factor	OR (95% CI)	*P*
BMI <30 kg/m^2	Reference group	
BMI ≥30 and <40 kg/m^2	1.49 (1.05–2.10)	0.024
BMI ≥40 kg/m^2	1.62 (1.13–2.33)	0.009
pT1a	Reference group	
pT1b	0.49 (0.34–0.73)	<0.001
pT2	0.58 (0.34–1.00)	0.048
pT3 or pT4	0.51 (0.30–0.88)	0.015
FIGO 1 endometrioid	Reference group	
FIGO 2 endometrioid	0.69 (0.48–0.99)	0.044
FIGO 3 endometrioid	0.76 (0.43–1.33)	0.331
Serous	2.16 (1.00–4.66)	0.049
Carcinosarcoma	1.74 (0.76–4.03)	0.192
Other histology[a]	0.70 (0.41–1.19)	0.185
pN0	Reference group	
pN1	0.76 (0.40–1.45)	0.404
pN2	0.74 (0.36–1.51)	0.404
pNX	1.03 (0.69–1.53)	0.902
Age	0.95 (0.71–1.29)	0.757

NOTE: The reference group for each factor is labeled.
[a]Other histologies include clear cell carcinoma, mixed epithelial carcinoma, and dedifferentiated or undifferentiated carcinoma.

Survival

Of note, 891 patients (99.7%) and 888 patients (99.3%) had death and recurrence data available for review, respectively, with 2.95 median years (range, days to 7.31 years) and 2.71 median years (range, days to 7.22 years) of follow-up time for death and recurrence data, respectively. Of the 3 patients excluded from the OS analysis, 2 were excluded due to missing data. The third excluded patient may have died of a simultaneous high-stage rectal adenocarcinoma. Of the additional 3 patients excluded from the recurrence analysis, 2 patients had distant metastases (brain and lung) at the time of diagnosis and 1 patient was not optimally debulked.

Overall survival

Of the patients with data available for analysis, 127 patients were deceased (14.2%) from any cause. Median time to death could not be calculated due to the low frequency of the event. High IGF1R expression patients had significantly higher OS compared with the low IGF1R expression group, $P = 0.002$, log-rank test. The Kaplan–Meier survival plot for OS by IGF1R expression is included as Fig. 3A. Univariate analysis (Cox proportional hazard model) revealed that the HR was 42% lower for patients with high IGF1R expression (HR, 0.58; $P = 0.002$). The multivariable model revealed that IGF1R expression remained significant after adjusting for pT, pN, and histology. BMI was not included in the multivariable model as it was not significant after adjusting for the other variables.

Died of disease

Seventy nine (8.9%) of the patients were dead of disease (DOD). A greater percentage of patients were DOD in the low IGF1R expression group (10.98%, $n = 38$) compared with the high IGF1R expression group (7.5%, $n = 41$). IGF1R expression was associated with DOD in this cohort, $P = 0.049$, log-rank test. Median time to death due to disease again could not be calculated due to the low frequency of the event. The association between IGF1R expression and DOD status was also seen in the univariate COX proportional hazard model with an HR of 0.64, $P = 0.051$. Having 79 deaths due to disease limited the number of covariates able to be included in a multivariable model. Univariately, pT, pN, and histology were significantly associated with DOD.

Disease-free survival

Of the patients with recurrence data available for analysis, 110 patients (12.37%) had recurrences, occurring predominantly in the vaginal cuff and pelvic and abdominal cavities. There was a significant difference between DFS curves between patients with low and high IGF1R expression, $P = 0.020$, with the high expression group showing improved DFS. Median time to recurrence was not calculated due to the low frequency of the event. A

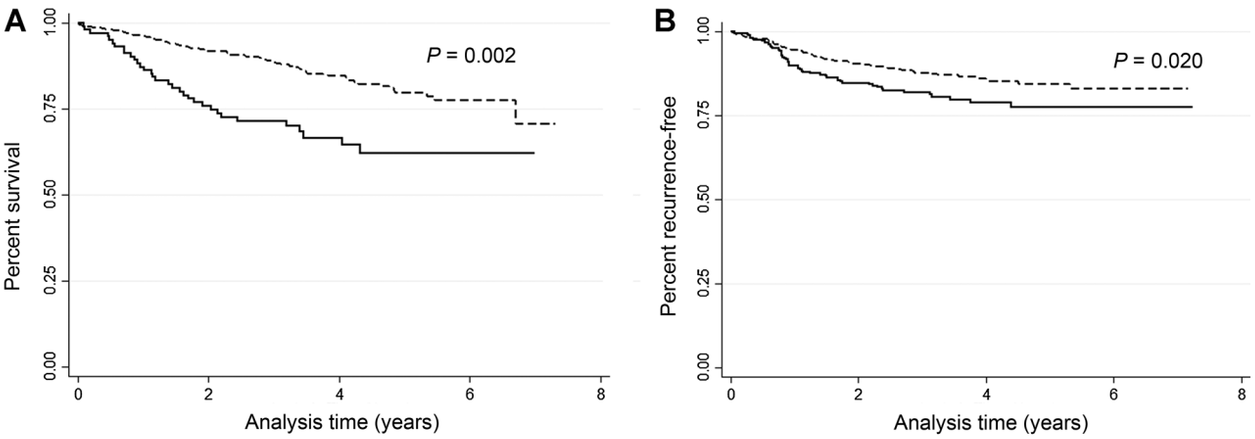

Figure 3.
OS (A) and DFS (B) curves for patients with high IGF1R expression (dashed lines) and low IGF1R expression (solid lines).

Kaplan–Meier survival plot for DFS by IGF1R expression is illustrated in Fig. 3B. Univariate analysis (Cox proportional hazard model) revealed the HR was 35% lower for patients with high IGF1R expression (HR 0.65, $P = 0.024$). The multivariable model revealed that pT, pN, and histology were significant factors, but IGF1R expression was not statistically significant after adjusting for these variables.

Discussion

IGF1R tumor expression demonstrated significant associations in our large cohort of EC. The proportion of tumors with high IGF1R expression (IRS 4–9) increased with BMI, our primary variable of interest. Indeed, the unadjusted odds of having high IGF1R expression was 1.79 (95% CI, 1.29–2.50; $P = 0.001$) for morbidly obese patients (BMI $\geq$40 kg/m^2) and 1.51 (95% CI, 1.08–2.10; $P = 0.015$) for obese patients (BMI $\geq$30–<40 kg/m^2) compared with patients having BMIs <30 kg/m^2. The ORs remained significant after adjusting for pT, pN, age, and histologic subtype. Furthermore, only comparisons between FIGO grade 1 and FIGO grade 2 endometrioid tumors and between FIGO grade 1 endometrioid tumors and serous tumors were borderline significant ($P = 0.044$ and $P = 0.049$, respectively) in a multivariable model that adjusted for BMI, age, pT, and pN indicating similar IGF1R expression across most of the histologic spectrum. Similar IGF1R associations in EC subtypes that are known to have widely different molecular bases and biologic potential (29, 30) may highlight physiopathologic commonalities linking obesity to EC in general. In fact, large epidemiologic studies have recently indicated that obesity is a risk factor for EC in general, albeit the association is stronger with low-grade tumors (2, 31, 32).

Interestingly, both univariate and multivariable results for pT status demonstrated significantly lower odds of having a high IGF1R expression for pT1b, pT2, or pT3 or pT4 compared with pT1a. Univariate comparison of high or low IGF1R expression between patients with positive and negative lymph nodes demonstrated that patients with pN1 and pN2 had lower odds of having high IGF1R expression when compared with pN0 patients, a finding that did not remain significant on multivariable analysis. In addition, our patients with high IGF1R expression also had a better DFS ($P = 0.020$) and OS ($P = 0.049$), the latter of which was shown by multivariate analysis to maintain significance after adjusting for pT, pN, and histology. With DFS, however, we could not demonstrate independent significance after accounting for other clinical factors. EC survival association with IGF1R expression has been reported only on a previous study by Piero and colleagues (24). These investigators reported a slightly longer survival time in patients with low level IGF1R expression (<10% of cells). However, their study included only 89 selected EC, disease-specific survival was not specified, and the survival association was not independently significant after standard clinicopathologic factors were taken into account (24). Studies in other tumors, including breast (33), lung (34, 35), oral (36), and laryngeal (28) carcinomas as well as synovial sarcomas (37), and melanocytic neoplasms (38), have indicated that higher IGF1R expression is associated with more aggressive clinical behavior. High IGF1R expression may have different pathologic and prognostic implications in different neoplasms, especially since obesity differentially influences cancer risk (39). In EC, reduced IGF1R expression may serve as a marker of hormone independence, associated with more aggressive EC and worse prognosis. The direct associations we have described herein between IGF1R expression, elevated BMI, lower pT, and better DFS and OS may help explain multiple other large studies showing higher BMI to be associated with more indolent, less aggressive pathologic features of EC (40–43).

EC IGF1R tissue studies, including the one by Piero and colleagues (24) previously discussed, are scant, include relatively small numbers of patients, have used different methodologies, and have produced inconsistent results. IGF1R was demonstrated in EC as early as 1990 when Talavera and colleagues (26) reported increased iodinated IGF-1 binding to isolated cell membranes of EC compared with non-neoplastic endometria. Maiorano and colleagues (22) and Roy and colleagues (25) showed similar IGF1R mRNA in EC compared with nonneoplastic endometria. Later, Hirano and colleagues (23) demonstrated IGF1R transcripts in most of 46 EC. Interestingly, although transcripts of two of the ligand-binding proteins (IGF-binding proteins, IGFBP 2 and 3) were also present in a majority of tumors, IGFBP-1 was detected only in a minority, possibly indicating a complex autocrine regulation (23). Similarly, Rutanen and colleagues (44) had detected no IGFBP-1 transcripts in their study of 20 EC. Finally, McCampbell and colleagues (21) reported increased IGF1R immunohistochemical detection and mRNA in 18 low-grade EC

compared with six normal endometria. Increased phosphorylated IGF1R was detected in the low-grade EC, consistent with activation of the receptor (21).

Our cohort is ideal to explore the IGF1R associations reported here because of its size, a majority of cases including lymph node dissections (84.1%) and the high prevalence of obesity and morbid obesity. However, there are several limitations. Follow-up times were relatively short with a median of 2.95 and 2.71 years for OS and DFS, respectively. Also, their relative infrequency precluded individual analysis of clear cell carcinomas, undifferentiated/dedifferentiated carcinomas and mixed epithelial carcinomas. In addition, the use of a TMA with one to three cores per tumor constitutes a potential limitation, especially given the observed tumor heterogeneity of IGF1R IHC staining. However, Spearman correlation coefficient showed strong association (0.757, $P < 0.001$) and weighted kappa statistic showed moderate correlation (0.44; 95% CI, 0.29–0.59) when whole-section IGF1R IHC was performed on a sample of tumors represented by a single TMA core and results were compared with those obtained with the TMA. Of note, in view of staining heterogeneity a decision was made to use maximum IRS among duplicate or triplicate TMA cores because it was expected to potentially represent a more applicable marker.

In summary, we contribute the largest EC series to date correlating IGF1R tumor expression with clinicopathologic data. Increased IGF1R expression was associated with higher BMI, pT1a tumors, and better OS and DFS. In addition, along with evidence from large epidemiologic studies (2, 31, 32) our current findings provide evidence that imbalances related to obesity and metabolism influence EC in general, and that the "type I vs. type II" approach to EC (45) is in need of new perspectives.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.S. Joehlin-Price, D.E. Cohn, A.A. Suarez
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.S. Joehlin-Price, J.A. Stephens, J. Zhang, D.E. Cohn, A.A. Suarez
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