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Expression of the Oncofetal Protein IGF2BP3 (IMP3) in Endometrial Clear Cell Carcinoma: Assessment of Frequency and Significance

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Abstract

Insulin-like growth factor-II mRNA-binding protein 3 (IGF2BP3 or IMP3) is a biomarker whose expression has been found to be a negative prognostic factor in several neoplasms, including ovarian clear cell carcinoma. In this study, we analyzed the frequency and clinicopathologic significance of IMP3 expression, as assessed by immunohistochemistry and as scored using a modified H-score system, in a cohort of 50 endometrial clear cell carcinomas (CCC). Cases with scores of 0-100, 101-200, and 201-300 were classified as negative/mildly positive (n=17), moderately positive (n=20) and strongly positive (n=13), respectively. A distinctive pattern of increased staining at the myoinvasive front (relative to the main tumor) was evident in 46% of the cases with evaluable foci of myometrial invasion. Moderate/strong IMP3 staining was associated with a tumor architectural pattern that has been reported to be of poor prognostic significance: at least 10% of the tumor composed of solid architecture or individual infiltrating tumor cells (p=0.01). Increasing levels of IMP3 expression showed a trend towards decreasing RFS (median survival 75.6, 81.3 and 48.4 months for the negative/mildly, moderately and strongly positive groups respectively (p=0.09). However, IMP3 expression was not significantly associated with reduced overall survival or RFS in a multivariate analytic model. The finding in a subset of our cases of increased IMP3 expression at the tumoral myoinvasive front is consistent with a role for IMP3 in invasiveness, as is the trend towards reduced RFS in cases expressing IMP3 at high

levels. These preliminary findings suggests that IMP3 expression may be involved in the pathogenesis of CCC, and is worthy of further exploration.

INTRODUCTION

The insulin-like growth factor-II mRNA-binding proteins IMP1 (IGF2BP1), IMP2 (IGF2BP2) and IMP3 (IGF2BP3) are an evolutionarily conserved family of mRNA-binding proteins that are comprised of two RNA-recognition motifs and four K-homology (KH) domains (1). IGF2BPs are involved in the subcytoplasmic localization of mRNAs, and hence translational control, during embryogenesis (2,3). Accordingly, they are primarily produced during the early stages of embryogenesis but may be expressed in some organs (such as the ovaries and testes) later in development (1,2). The IGF2BP3 gene is located on chromosome 7p11.5, was previously designated as KOC (K homology domain containing protein overexpressed in cancer, and was cloned following a screen for differentially expressed genes in pancreatic cancer relative to the normal pancreas (4). The resultant protein, a 580-amino acid oncofetal RNA binding protein (IMP3), has emerged during the last decade as a robust diagnostic marker whose immunohistochemical assessment can be utilized to reliably distinguish some malignancies from their benign histologic or cytologic mimics, and to distinguish between histotypes of selected neoplasms (5). Furthermore, preliminary lines of evidence indicate that IMP3 expression is a negative prognostic factor and/or a predictor of tumor progression in a remarkably high proportion of neoplasms in which this expression has been evaluated, including colorectal, gastric, renal, mammary, bladder, thyroid and oral cavity carcinomas, cutaneous melanomas, osteosarcomas, meningiomas, neuroblastomas, and cervical intraepithelial neoplasias (5). It was recently reported that IMP3 expression is an independent marker of reduced survival in patients with ovarian clear cell carcinomas (6). Endometrial clear cell carcinoma (CCC) is a significantly rarer neoplasm, and a comparable analysis of IMP3 expression in CCC has not heretofore been reported. The purpose of this study is to assess the frequency and prognostic significance of IMP3 expression in endometrial clear cell carcinomas.

MATERIALS AND METHODS

Case Selection and Review

The 50 cases that comprised the final dataset were retrieved from the archived files of multiple institutions. The cases were selected at these institutions by gynecologic pathologists, who all searched for tumors signed out as CCC at their respective institutions, subjected them to secondary review, and selected those cases that they considered to be unequivocally diagnostic of CCC. All cases were subsequently reviewed centrally by a panel of 3 gynecologic pathologists (JH, VP and OF). Each panelist reviewed all cases independently. A case was included in the final dataset only if at least 2 of the 3 central panelists agreed with a diagnosis of CCC. 19% of the cases that were in the pre-review dataset were excluded as non-CCC after central review. The 50 cases that comprised the final dataset thus included cases that had been subjected to at least three layers of review. These cases included 5 biopsies and 45 hysterectomy specimens. The cases had been used, entirely or in subsets, in previously published studies from this group. Clinical data were extracted from the medical record. This study was approved by the institutional review board (IRB) at Vanderbilt University (IRB #12606).

Immunohistochemistry

IMP3 immunohistochemical analyses were performed on a single section from all 50 cases in a Leica Bond Max immunohistochemical autostainer (Leica Microsystems, Buffalo

Grove, IL). The proprietary Leica Epitope Retrieval 2 solution was used to facilitate heat induced antigen retrieval. Slides were then incubated with the primary antibody, a monoclonal mouse anti-Human IMP3, Clone 69.1 (dilution 1:750, Dako, Carpinteria, CA); the Bond Polymer Refine detection system was used for visualization. IMP3 is known to exhibit a predominantly cytoplasmic reactive pattern, although nuclear expression may also be uncommonly seen. Any immunoreactivity (nuclear and/or cytoplasmic) was interpreted as representing positivity. A case of endometrial serous carcinoma with known IMP3 immunoreactivity served as positive control, whereas a section of normal endometrium in the proliferative phase served as a negative control. Immunohistochemical staining for IMP3 was jointly scored on all cases by 2 authors (OF and MMD) using a semi-quantitative system that is based on the H-index (7): $3 \times$ percentage of strongly staining cells + $2 \times$ percentage of moderately staining cells + percentage of weakly staining cells, giving “composite scores” that ranged from 0 to 300. (figure 1). Given the high frequency of immunoreactivity as outlined below, the cases were classified into three groups based on their composite scores. Cases with a score of 0 to 100 were interpreted as being negative/mildly IMP3-positive; cases with scores of 101 to 200 were interpreted as being moderately IMP3-positive and cases with scores of 201 to 300 were interpreted as being strongly IMP3-positive.

Statistical analysis

The 3 subgroups (IMP3 negative/mildly positive; IMP3 moderately-positive, and IMP3 strongly-positive) were assessed for differences in overall survival (OS) and relapse free survival (RFS) using log-rank tests. Using a dichotomous classification of IMP3 immunoreactivity (IMP3 negative/mildly positive versus IMP3 moderately-positive + IMP3 strongly-positive), the resultant subgroups were compared regarding a variety of clinicopathologic variables using Fisher exact, Pearson Chi square, and Student t tests depending on the type of variable being analyzed. These tests were also applied to the entire dataset assessing for any significant univariate associations between the following variables on OS or RFS: patient age (>65 versus ≤ 65 years), FIGO stage, predominant architectural patterns, lymph node status, distant metastases, lymphovascular invasion, mitotic index, myometrial invasion (>50 versus $\leq 50\%$), architectural grade and IMP3 immunoreactivity. Multiple regressions for variables showing a significant bivariate association with survival were performed using a Cox regression model. A 2-tailed p value of <0.05 was considered to be statistically significant for all analyses.

RESULTS

Patients

The 50 patients ranged in age from 50 to 85 (mean 67.8; median 67). Their stage distribution, using the criteria of the International Federation of Gynecology and Obstetrics and the American Joint Cancer Committee staging systems, was as follows: stage I (n=19, including one patient with no residual disease in hysterectomy specimen: pT0, IA; n=18; IB, n=1), stage II (n=8), stage III (n=14; IIIA, n=6; IIIB, n=1; IIIC, n=7), stage IV (n=9). All but 2 patients underwent a total hysterectomy with bilateral salpingo-oophorectomy; lymphadenectomy was performed in 43 patients (pelvic nodes only in 10, both pelvic and paraaortic in 23). In 10 patients lymph nodes were positive for metastatic disease. The patient population was fairly heterogeneous with regard to adjuvant treatments, which included chemotherapy and adjuvant radiotherapy (n=9), radiotherapy only (n=12), and chemotherapy only (n=10); 1 patient received chemotherapy only without surgical resection; 1 received neoadjuvant chemotherapy, surgery and adjuvant chemotherapy. 7 patients received no further treatment post-surgery, and adjuvant management is unknown in 10. Follow-up was available in 43 patients (median duration 31 months); 25 were without

evidence of disease, 9 were dead of disease, 8 were alive with disease, and 1 was dead of other causes. There were 11 relapses, occurring 1 to 27 months (mean 11.2 months) after primary surgical resection. Relapse sites were in the vagina (n=2), pleura (n=1), inguinal/groin region (n=2), supraclavicular lymph node (n=1), kidney (n=1), bone (n=2), abdominal soft tissue (n=1), and lungs (n=1).

Immunoreactivity Patterns

IMP3 expression was entirely negative in 5 cases, but the remaining 45 cases showed varying degrees of IMP3 immunoreactivity: 12 cases had a score of between 1 and 100 (weakly positive), 20 had scores that were between 101 and 200 (moderately positive), and 13 cases had scores that were between 201 and 300 (strongly positive). Subcellular localization was predominantly cytoplasmic in all IMP3-positive cases. In 17 (46%) of the 37 cases in which myometrial invasion was directly evaluable in the stained section, a distinctive pattern of increased staining at the myoinvasive front (relative to the main tumor) was evident (figure 1B). In the other 18 cases, the tumor nests at the myoinvasive front either showed the same staining intensity as the main tumor, or displayed an identical level of heterogeneity. In no case were the tumor nests at the myoinvasive front less immunoreactive than the main tumoral mass. The background, non-neoplastic endometrium and myometrium were entirely IMP3 negative in all evaluable cases. Foci of clear cell intraepithelial carcinoma (8,9) showed an identical pattern and intensity of immunoreactivity as the adjacent tumor in all 9 cases in which they were evaluable.

IMP3 Expression and Clinicopathologic Variables

Of the 43 patients with follow-up, there were 12 and 31 tumors in the IMP3 negative/weakly positive and IMP3 moderately/strongly positive groups respectively. There was no statistically significant differences between the IMP3 negative/weakly positive group and IMP3 moderately/strongly positive group regarding most of the variables on table 1, which included patient age, FIGO stage, frequency of lymphovascular invasion, tumoral mitotic index, rate of recurrences, and rate of lymph node metastases. The IMP3 moderately/strongly positive group had a comparatively higher frequency of cases with >30% myometrial invasion ($p=0.03$), a significance that disappeared when the analysis was repeated at the 50% myometrial invasion threshold. There was also an apparent association between IMP3 moderate/strong immunoreactivity and the architectural grading system that has recently been proposed by Yamamoto et al (10) for ovarian clear cell carcinoma. This system is based only on tumoral architecture, and classifies CCC into 3 subgroups: Grade A tumors have 90% of a tumor composed of well-differentiated tubulocystic and/or papillary patterns; Grade C tumors have at least 10% of the tumor composed of solid masses or individual infiltrating tumor cells; Grade B tumors do not fit either of the aforementioned descriptions (10). To facilitate our analysis given the size of our dataset, we combined grades A and B cases and compared them with Grade C cases; the latter were reported by Yamamoto et al to represent the prognostically worst group (10). We found that 89% of the architecturally grade C cases were moderately to strongly IMP3 positive, as compared with 54.8% of the group A+B cases ($p=0.01$). On univariate analyses, IMP3 expression was not found to be associated with reduced overall survival at any definitional threshold for positivity (Figure 2A, table 2). However, the IMP3 negative/weakly positive, IMP3 moderately positive, and IMP3 strongly positive groups showed a trend towards decreasing RFS (median PFS 75.6, 81.3 and 48.4 months respectively), although the differences approached but did not attain statistical significance ($p=0.09$) (figure 2B). On a multivariate analytic model that included the aforementioned variables, IMP3 expression was not significantly associated with reduced OS or RFS.

DISCUSSION

IMP3 is not expressed in most adult tissues but can be re-activated in a variety of cancers, hence its designation as an oncofetal protein (2). In the uterus, IMP3 is commonly expressed in decidualized stromal cells (gestational and non-gestational) and is variably expressed weakly in endometrial epithelia. Non-decidualized endometrial stromal cells, myometrium and uterine endothelial cells are all IMP3 negative (11). IMP3 expression has been found to be of significant diagnostic utility in the uterus, including in the distinction of cervical adenocarcinoma in-situ and mucinous minimal deviation adenocarcinoma from benign endocervical glands (12,13), the distinction of uterine leiomyosarcomas from leiomyomas (14), and in predicting the small subset of cervical intraepithelial neoplasia with an increased likelihood of progression (15). IMP3 expression has also been reported to vary by endometrial carcinoma histotype, with the highest frequency of expression in serous carcinomas, where 98 to 100% of cases have been reported to be IMP3-positive (11,16,17). Reported IMP3-positivity rates in endometrioid carcinomas have generally been low but have been somewhat variable: three studies reported that 7%, 7%, and 56% of cases were IMP3-positive (11,16,17); in the latter study, however, 73% of the IMP3-positive cases had immunoreactivity that was limited to 20% of tumor cells (16). There have only been 2 studies evaluating IMP3 expression in endometrial CCC: Zheng et al (11) reported that 11 (61%) of 18 cases displayed at least focal IMP3 expression and Alkushi et al (18) reported that 3 (60%) of 5 cases were IMP3-positive. Neither study, however, was aimed at deciphering the prognostic significance of IMP3 expression. Given the aforementioned report of IMP3 expression being associated with reduced survival in ovarian CCC (6), we analyzed the frequency and significance of IMP3 expression in a cohort of endometrial CCC. Our study shows that IMP3 expression is very frequent (90% positivity rate) in endometrial CCC, and that high levels of expression is associated with reduced RFS and an infiltrative or solid architectural pattern (the recently described Yamamoto et al (10) architectural grade C). We have previously shown in this dataset that the aforementioned architectural grade 3 is associated with reduced survival on univariate but not multivariate analyses (19). The association of IMP3 expression with solid architectural pattern is noteworthy, since this pattern to varying extents have been reported to be a negative prognostic factor in ovarian CCC (10,20,21). Nevertheless, it is unclear if the noted associations are independent of each other or other variables given the results of our multivariate model. There are two major limitations to this study, and our findings should be evaluated within the context of these limitations. The first are related limitations introduced by the rarity of the histotype, which is an impediment to the analysis of a larger dataset and the validation of any staining thresholds on independent datasets. Second, our patients received a wide variety of adjuvant treatments, resulting in small treatment subgroups that preclude meaningful subsidiary analyses of the significance of IMP3 expression in each of the various treatment groups. As such, our findings are preliminary in nature.

The diversity of tumor types and anatomic locations in which IMP3 expression has been found to be of poor prognostic significance suggests that this protein plays a fundamental role in tumorigenesis and/or progression that is neither tumor type nor organ-specific. Multiple lines of preclinical evidence suggest that this role is related, at least partially, to its facilitation of invasiveness (21). In hepatocellular carcinomas (HCC), IMP3 expression is accentuated at the tumoral invasive front and this protein is expressed at higher levels in metastatic nodules than in the primary tumors (23); similar findings have been reported in a glioblastoma mouse model (24). Furthermore, a significant decrease in cell motility and invasion can be elicited in HCC cell lines by depleting IMP3 (23), and IMP3 knockdown of leukemia, glioblastoma, cervical cancer, breast cancer, and melanoma cell lines all eventuate in markedly reduced cell migration and invasiveness (15,24-27). The precise mechanistic basis for the oncogenic manifestations of IMP3 remain unclear, but probably involves some

combination of translational activation of IGF-2 (and activation of IGF2 downstream effectors) to promote cellular proliferation and/or cellular survival (27-29), and the promotion of cancer migration and invasion through the binding of invasion-related mRNAs, including CD44, CD164 and matrix metalloproteinase 9 (27,28). The finding in a subset of our cases of increased IMP3 expression at the tumoral myoinvasive front, is consistent with a role for IMP3 in invasiveness, as is the trend towards reduced RFS in cases expressing IMP3 at high levels.

In summary, IMP3 is expressed in a high proportion of CCC but at varying levels. High levels of IMP3 expression may be associated with reduced RFS but may co-vary with other factors, including architectural patterns. Its high frequency of expression, in conjunction with previously reported findings (11,16,17), suggest that it is involved in the pathogenesis of type II endometrial carcinomas, and is worthy of further exploration.

Acknowledgments

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Figure 1.

A. Morphologic features of a case of clear cell carcinoma (hematoxylin and eosin; original magnification $\times 200$),

B. Accentuation of IMP3 immunoreactivity at the myoinvasive front of the tumor (immunoperoxidase; original magnification $\times 100$),

C-F. Intensity component of scoring system for IMP3 immunoreactivity: C (no staining), D (weak staining), E (moderate staining), F (strong staining) (immunoperoxidase; original magnification $\times 200$ for figures 1C and 1E; original magnification $\times 400$ for figures 1D and 1F),

Figure 2.
Kaplan-Meier survival curves showing overall (A) and relapse free (B) survival rates.

Table 1

Classification of cases based on IMP3 immunoreactivity patterns

PARAMETER	IMP3 weakly positive or negative	IMP3 moderately or strongly positive	P VALUE
<i>Number of cases</i>	17	33	
<i>Age (years)</i>	67.6	67.9	0.9
<i>Stage at Presentation (n)</i>			
Uterus Confined disease (FIGO stage I and II)	11	16	0.37
Extrauterine Disease (FIGO stage III and IV)	6	17	
<i>Lymphovascular invasion present (n)</i>	7	15	0.8
<i>Myometrial invasion</i>			
Average (%)	7.6	12.3	0.25
50% (n)	10	19	1
>50% (n)	7	13	
Unknown	0	1	
<i>Lymph nodes obtained and status known (n)</i>	13	27	
positive	2	8	0.46
negative	11	19	
<i>Mitotic Index</i>	4	4.7	0.7
<i>Architectural Grade^b</i>			
A and B	14	17	0.01
C	2	17	
<i>Relapses/Recurrences^a</i>			
Number of cases with follow-up	12	31	0.23
No of cases with relapses/recurrences	1	10	

^aFor cases with follow-up^bArchitectural grade by Yamamoto et al criteria (10)

Table 2

Association between Clinicopathologic factors, including IMP3 expression, and Relapse Free Survival

Parameter	Number of patients	Survival (months) Median ± SE	95% confidence interval	P value (univariate)	P value (multivariate)
IMP3 composite score					
0-100 (negative-mildly positive)	12	75.6 ± 7	61.8 – 89.4	0.09	NS
101-200 (moderately positive)	17	81.3 ± 11.7	58.4 – 104.5		
>200 (strongly positive)	14	48.4 ± 13.5	22.1- 74.9		
Age					
> 65 years	25	70.6 ± 9.3	52.2 – 88.8	0.02	0.023
65 years	18	75.6 ± 10.7	54.7 – 96.5		
FIGO stage					
I and II	26	91.2 ± 7.1	77.3 – 104.8	0.002	NS
III and IV	17	16.5 ± 2.0	12.5 – 20.5		
Architectural pattern In >50% of tumor					
Glandular	17	71.8 ± 10.5	51.2 – 92.5	NS	NS
papillary	12	64.4 ± 15.8	33.3 – 95.4		
Solid	10	37.4 ± 3.4	30.5 – 44.1		
cystic	4	60.3 ± 18.5	24.1 – 96.6		
Lymph nodes					
positive	8	13.7 ± 2.8	8.2 - 19.1	0.002	NS
negative	29	84.1± 8.1	96.1- 101.4		
Lymphovascular Invasion					
positive	20	70.1 ± 11.1	48.4- 91.8	NS	NS
negative	22	76.4 ± 8.9	58.9-93.9		
Myometrium invasion					
>50%	19	57.2 ± 9.3	38.1 – 76.4	NS	NS
50%	24	81.3 ± 10.4	62.7 – 98.5		
Unknown	1				
Mitotic index					
>4	12	68.9 ± 13.4	42.7 – 95.2	NS	NS
4	31	77.3 ± 7.6	61.3 – 91.2		
Architectural grade ^a					
A+B	24	77.1 ± 9.4	58.6 – 95.5	0.09	NS
C	19	32.6 ± 3.6	25.6 – 39.6		

NS: not statistically significant (all p values between 0.05 and 0.09999 are listed); SE standard error; FIGO international Federation of Gynecology and Obstetrics.

^a Architectural grade per Yamamoto et al criteria (10)