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Positive expression of insulin-like growth factor-1 receptor is associated with a positive hormone receptor status in ovarian cancer

L.Q. Wang[1,2], W.D. Zhao[1], X.J. Jiao[2], D.L. Du[2], Y.S. Liu[1]

[1] Department of Obstetrics and Gynecology, Provincial Hospital, Affiliated to Anhui Medical University, Hefei
[2] Department of Obstetrics and Gynecology, The First Affiliated Hospital of Bengbu Medical College, Bengbu (China)

Summary

Purpose: Ovarian cancer is the most deadly of all gynecologic malignancies, due in part to the diagnosis at an advanced stage caused by the deficiency of specific marks and symptoms, by the absence of reliable tests for screening, and by early detection. *Materials and Methods*: Insulin-like growth factor-I (IGF-I) is known to be involved in the development and promotion of diverse examples of solid tumors including ovarian cancer. IGF-I levels in local tissue are subject to both endocrine and paracrine/autocrine regulation. *Results*: Most patients will react initially to treatment, but almost 70% of them will have a recurrence. Consequently, new therapeutic modalities are urgently required to overcome chemoresistance observed in ovarian cancer patients. IGF-1R expression was evaluated immunohistochemically in tissue microarray blocks constructed from 1,200 ovarian cancer samples collected from three medical institutions. *Conclusion*: Evidence accumulates suggesting that the insulin/insulin growth factor (IGF) pathways could play a good therapeutic target in various cancers, including ovarian cancer.

Key words: Insulin-like growth factor-1 receptor (IGF1-R); Ovarian cancer; Immunostaining.

Introduction

Carcinomas arising from the epithelial cells of the ovary are the fifth most common malignancy in women and the leading cause of death from gynecological cancers. Epithelial ovarian cancers comprise a group of linked but distinct carcinomas that likely originate from a common epithelial cell type, but develop via differentiation pathways and differ in their clinical presentation and etiology. They are currently separated into different histological subtypes (including serous, endometrioid, mucinous, and clear cell) based on their morphological resemblance to normal epithelia in the gynecological and intestinal tracts; however the genetic basis underlying their divergence is poorly understood [1, 2].

The insulin-like growth factor-1 receptor (IGF1-R) is a cellular receptor overexpressed in many tumour cell lines and in some human tumours that seem to play a critical role in transformation, tumourigenicity, and metastasis. An overexpression and enhanced activation of IGF-1R are seen in many malignant tumors, including ovarian cancer [3]. IGF-1R is a glycosylated heterotetramer composed of two extracellular α-subunits and β-subunits that have intrinsic tyrosine kinase activity with 70% homology of the insulin receptor. IGF-1R mainly mediates the effect of insulin-like growth factors (IGFs), which are potent mitogens that regulate cell proliferation, differentiation, and protection from apoptosis [4, 5]. It acts as an significant part in the formation and maintenance of the transformed phenotype. It too bears a potent anti-apoptotic activity and sustains a important influence on the control of cell and body size. Down-regulation of the IGF-1R leads to massive apoptosis of malignant neoplastic disease cells. These features make it an attractive target for anticancer therapy [5]. IGF1R signalling interferes with numerous growth factors and receptors such as VEGF and EGFR. The IGF1 receptor (IGF1-1R) has been shown to be expressed in a wide range of tumours, and IGF1R signalling is crucial for tumour transformation and the survival of malignant cells [6]. The binding of the ligand (IGF1 or IGF2) to this receptor activates multiple transducing pathways linked to different cellular functions, including, among others, transformation, invasiveness, metastatic potential, and protection from apoptosis [7-10]. The molecular events induced by estrogens via estrogen receptor in these cancers are mediated in genomic or non-genomic signal transduction pathway [11]. Testing of multiple cases of tumors shows an abundant expression of IGF-IR, suggesting that up-regulation of the IGF-IR gene constitutes a usual paradigm in cancer development [12].

Epithelial ovarian tumors, which are the majority of malignant ovarian tumors, are further grouped into histological types as follows: serous, mucinous, endometrioid, clear cell, transitional cell tumors (Brenner tumors), carcinosar-

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coma, mixed epithelial tumor, undifferentiated carcinoma, and others [13]. Epithelial ovarian cancer is the deadliest gynecologic malignancy, yet its molecular etiology remains poorly understood. Evidence is gathering to support a role in the insulin-like growth factor family in human carcinogenesis, and recently using microarray expression analysis. The majority of epithelial ovarian cancer (EOC) patients present with advanced-stage disease at the time of initial diagnosis and experience recurrence despite the excellent response rates observed with platinum-based chemotherapy (e.g., cisplatin). Disease recurrence typically occurs within two to three years after first-line chemotherapy and is accompanied by the development of cisplatin resistance [14]. Tumors of surface epithelial origin constitute about two-thirds of all ovarian neoplasms and an even larger proportion of ovarian malignant neoplasms. They occur predominantly in adults, with the malignant forms generally appearing later in life [15]. Thus, there is an urgent need to develop effective pharmacological agents for the treatment of EOC. Most patients with EOC will receive a satisfactory initial clinical response to aggressive cytoreductive surgery followed by combination chemotherapy, but alas, this will usually not lead to cure [16]. A cohort of 930 primary breast cancer patients, IGF-1R was expressed in 87% of the lawsuits were reported [17].

Topotecan is an active drug in platinum-sensitive ovarian cancer, with substantial but manageable hematologic toxicity. Topotecan has efficacy at least equivalent to paclitaxel manifested by the higher response rate and significantly longer time to progression [18, 19]. Presently, in this respect, is not a targeted treatment for triple-negative ovarian cancers, and patients stricken with this subtype usually die within two years of diagnosis.

Insulin-like growth factor receptor-1 (IGF-1R) and vascular endothelial growth factor-A (VEGF-A), which regulates angiogenesis are key agents in these footpaths. Despite the development of new therapeutic approaches, these survival statistics remained largely unchanged for many years. Clearly, there is a demand for better understanding the molecular pathogenesis of ovarian cancer, so that new drug targets or biomarkers that help early detection can be made out.

Materials and Methods

Patients and samples

Primary invasive ovarian carcinoma samples were collected-from three medical institutions. In total, 1,094 samples were obtained from the Second Affiliated Hospital of Bengbu Medical College (187 cases, 2010), the Third People's Hospital of Bengbu (167 cases, 2009-2011), and the First Affiliated Hospital of Bengbu Medical College (200 cases, 2010–2012). All tissues were surgically resected, fixed at 10% buffered formalin, and embedded in paraffin. A pathologist in each institution reviewed the slides and selected a representative block for each instance. The tumor area on the paraffin block was marked with a pen and sent to the Bengbu Medical College to construct tissue microarray blocks. Patient and tumor characteristics, including age, type of surgery, histological type, histological grade, and follow-up information were received from the aforementioned establishments.

Immunohistochemical staining

Immunoperoxide staining was carried out using the supersensitive streptavidin-biotin detection kit or the universal avidin-biotin DAB detection system. Slides were counterstained with Mayer's hematoxylin. To assess the specificity of the antibodies, known positive and negative tissue controls were applied. The answers were reported as histoscores derived from the formula: % positive cells (1+ staining intensity). Tumors with histoscores greater than or equal to 10 were considered positive for the marker in question.

Silver-enhanced in situ hybridization (SISH)

Four-micrometer-thick sections were immunostained with primary antibodies against ER (diluted 1:50, estrogen receptor alpha mouse anti-human monoclonal antibody), estrogen receptor alpha mouse anti-human monoclonal antibody, and IGF-1R (diluted 1:100, G11). SISH was performed using a SISH detection kit and chromosome 17 (Chr17) probes on two consecutive TMA sections using a benchmark automatic immunostaining device according to the manufacturer's protocol. Both probes were labelled with dinitrophenol (DNP).

The *HER2* DNA probe was denatured at 95°C for 12 minutes and hybridization was performed at 50°C for two hours. After hybridization, appropriate rigour washes were performed three times at 70°C. The Chr17 probe was denatured at 90°C for 15 minutes and hybridization was performed at 45°C for 90 transactions. After hybridization, appropriate rigor washes were done three times at 50°C. The *HER2* and Chr17 DNP-labeled probes were visualized using rabbit anti-DNP primary antibody with an ultraview SISH detection kit. Silver precipitated in the nuclei following the sequential addition of silver acetate, hydroquinone, and H_2O_2. The slides were counterstained with hematoxylin to facilitate interpretation by light microscopy.

Interpretation of immunohistochemical staining and SISH data

The interpretation of data in the present experiment was carried from a previously reported study for the SISH data for *HER2* expression and immunohistochemistry data [20]. The results from the SISH was carried according to [20]. In short, the signals from the *HER2* and Chr17 were calculated in over 20 non-overlapping nuclei per sample. The data was interpreted and confirmed by the American Society of Clinical Oncology and the College of American Pathologists guidelines [21]. All the immunohistochemical markers were assigned a positive or negative grade. The estrogen was assessed according to the Allred score. A tumor was considered positive for ER or PR when the total score was >2. The strength of the staining within the invasive tumor component in accordance with the HER2 expression scoring system is described in the Hercep TestTM manual; the IGF-1R expression was confirmed using the membrane staining. The result was defined as positive (3+) when the tumor presented a uniform, intense membrane staining in more than 30% of the invasive tumor cells. An equivocal (2+) result was determined as the presence of light or non-uniform membrane staining in more than 10% of the tumor cells [22]. A negative result was defined as the presence of weak/incomplete membrane staining (1+) or the perfect absence of membrane staining (0) in any part of the tumor cells (Figure 1). Heterogeneous staining of IGF-1R within any area of the tumour was assigned the highest score. An IGF-1R score of 2+ or 3+ was considered positive. The immunochemical substituted panel was made according to a gene expression for ovarian cancer types and designated: 1). Luminal A:

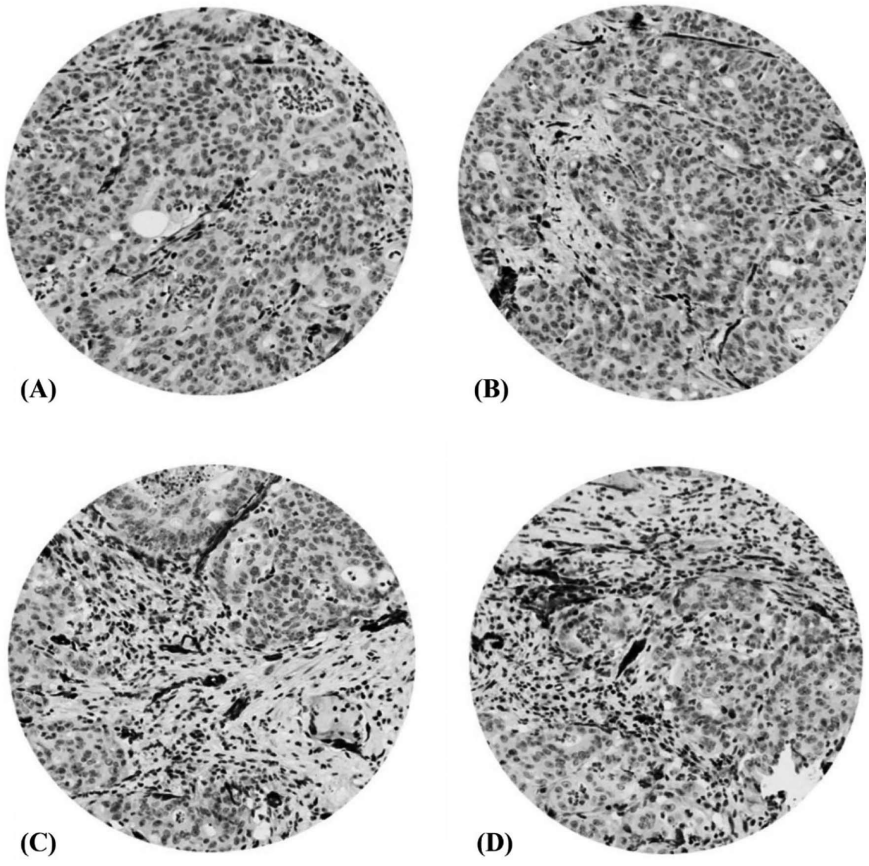

Figure 1. — Immunohistochemical staining of insulin-like growth factor 1 receptor (IGF-1R). (A) score = 0, (B) score = 1, (C) score = 2, (D) score = 3 (immunohistochemical staining, ×100).

ER+ and/or PR+ and HER2−, 2). luminal B: ER+ and/or PR+ and HER2+, 3). HER2: ER−, PR− and 4). Triple-negative: ER−, PR−, and HER2− (Control).

Statistics

Comparison of differences between histologic subsets were made using Student's *t*-test, with a two-tailed p value of < 0.05 considered to be significant. To estimate the overall survival (OS), patients were followed-up from the date of surgical excision of EOC until the date of death. Patients who were lost during follow-up or died from causes other than EOC were excluded from the analysis. The log rank test and the Cox proportional hazard model compared the survival curves of two or more groups with one another. All statistical analyses were done using the GraphPad Prism 6.

Results

Starting point clinical features

Of the 1200 invasive EOC samples collected, 1,020 samples were included in the last analysis. The remaining samples were removed due to non-informative scores or loss of scores while performing the IGF-1R immunostaining. The 1,020 EOC cases included in this survey contained 872 cases of serous cystadenocarcinoma, 72 cases of mucinous cystadenocarcinoma, 43 cases of endometrioid carcinoma, and 33 cases of carcinomas of other histological types. The average patient age was 46 years (range, 20-80 years) and the median follow-up period was 84 months. In total, 228 patients experienced relapse or death (19.0%) and 154 patients (12.9%). The average time between the date of diagnosis and the date of death was 41.2 months (range, 41 days to 92 months).

IGF-1R manifestation and its connotation with histological features

Positive IGF-1R expression was observed in 424 (35.4%) of the 1,200 samples. Of the 1020 samples that could be classified, positive IGF-1R expression was observed in 696 samples (68.2%). This subset included samples from all four subtypes. IGF-1R was frequently expressed in the luminal A and luminal B subtypes (82.1% and 71.4 %, respectively). Yet, just 51.1% of the HER2 subtypes were IGF-1R positive ($p < 0.001$). In the triple-negative subtype, 41.1% of the cases were IGF-1R positive (Table 1). Positive IGF-1R expression was linked with a positive hormone receptor status (for both ER and PR) and the absence of HER2 gene amplification ($p < 0.001$). In addition, IGF-1R positivity was associated with low histological grades ($p < 0.001$). There was no correlation between IGF-1R expression and age, T stage (tumor size), and the presence or absence of lymph node metastasis.

Table 1. — *Insulin-like growth factor 1 (IFG-1R) receptor expression in molecular subtypes.*

Molecular subtypes based on Immunohistochemistry (%)	Negative (n=451) No. (%)	Positive (n=910) No. (%)	*p*-value
Luminal A	88 (19.24)	413 (82.1)	< 0.001
Luminal B	25 (24.1)	60 (71.4)	
HER2	95 (81.3)	21 (51.1)	
Triple-negative	141 (51.21)	122 (41.1)	

HER2 = human epidermal growth factor receptor2.

Table 2. — *Correlation between insulin-like growth factor 1 receptor expression and histological features and hormonal receptor status.*

Clinical feature	Negative No. (%)	Positive No. (%)	*p*-value
Age (year)			0.251
<50	199 (59.4)	398 (67.8)	
≥50	123 (41.3)	234 (35.9)	
Histological grade			< 0.001
1	51 (15.9)	110 (23)	
2	162 (52.1)	351 (60.4)	
3	182 (7.2)	41 (6.2)	
T stage			0.261
T1	152 (52.4)	420 (57.1)	
T2	213 (51.2)	372 (50.2)	
T3	33 (7.52)	42 (4.2)	
LN status			0.294
Negative	220 (56.8)	372 (51.3)	
Positive	182 (49.2)	372 (42.1)	
ER			< 0.001
Negative	261 (75.2)	173 (28.3)	
Positive	173 (42.8)	330 (41.02)	
PR			< 0.001
Negative	271 (69.3)	573 (84.1)	
Positive	94 (42.1)	451 (63.1)	
HER2			< 0.001
No amplification	221 (67.3)	592 (84.4)	
Amplification	114 (31.8)	84 (14.2)	
Systemic therapy			< 0.001
None (placebo-control)	58 (15.2)	48 (6.1)	
Topotecan	41.4 (15.3)	162 (43.1)	
Chemotherapy	193 (52.5)	132 (12.4)	
Chemotherapy+ topotecan	73 (41.5)	362 (54.2)	

LN = lymph node; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

Table 3. — *Multivariate analysis of overall survival in all invasive epithelial ovarian cancer (EOC)*

Factor	Unfavourable	HR	95% CI	*p*-value
IGF – 1R	Negative	1.572	0.821−2.18	0.265
Histologic grade	3	1.721	0.712−3.211	0.215
T stage	T2	1.632	1.312−3.81	0.015
	T3	5.182	2.83−6.23	< 0.001
LN status	Positive	4.23	3.11−3.61	< 0.001
Subtype	HER2	2.33	1.42−5.21	0.020
	Triple-negative	1.92	0.42−2.81	0.528

HR = hazard ratio; CI = confidence interval;
IGF-1R = insulin-like growth factor 1 receptor; LN = lymph node;
HER2 = human epidermal growth factor receptor 2.

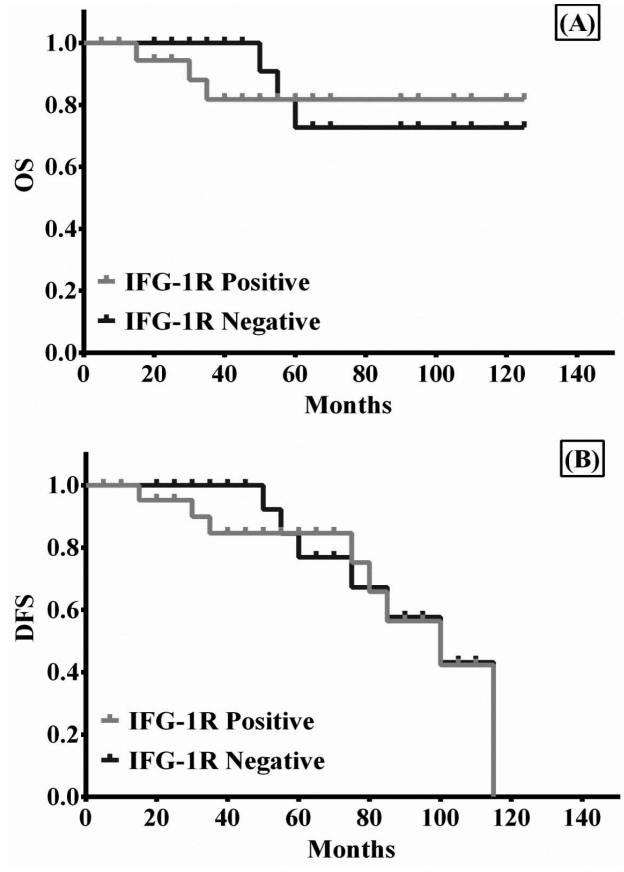

Figure 2. — Kaplan-Meier analysis of OS (A) and DFS in ovarian cancer. (B) IGF-1R = insulin-like growth factor 1 receptor.

IGF-1R expression and natural selection

The OS was significantly longer in ovarian cancer cases with positive IGF-1R expression than in those with negative IGF-1R expression ($p = 0.026$) (Figure 2A). However, IGF-1R expression did not correlate with disease-free survival (DFS) (Figure 2B). There was no correlation between IGF-1R expression and age, T stage (tumour size), and the presence or absence of lymph node metastasis (Table 2). In the multivariate analysis, a high T stage, positive LN status, and the HER2 subtype were statistically significant poor prognostic factors, while negative IGF-1R expression lost statistical significance (Table 3). In luminal A, luminal B, and HER2 sub-types, OS and DFS were not associated with IGF-1R expression. In the triple-negative subtype, DFS was significantly shorter in cases with positive IGF-1R expression ($p = 0.020$), but OS did not appear to be connected with the IGF-1R expression (Figure 3).

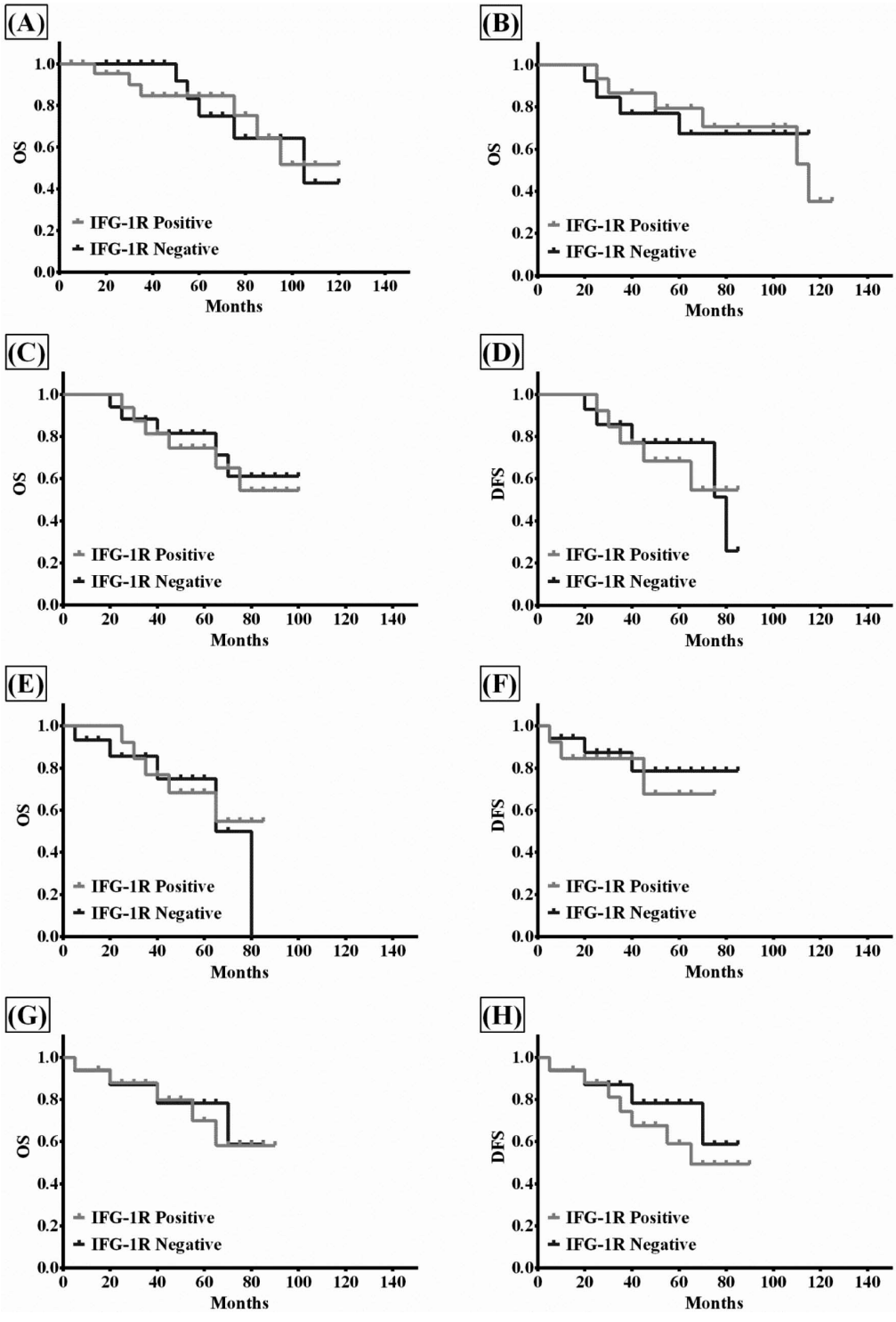

Figure 3. — Kaplan-Meier analysis of OS and DFS in EOC subtypes: luminal A (A and B), luminal B (C and D), HER2 (E and F), and triple-negative subtypes (G and H). IGF-1R = insulin-like growth factor 1 receptor.

Discussion

The ovarian surface epithelium is histologically similar to the mesothelium, which is the epithelium that lines the inside of the pelvic and abdominal cavities. This similarity, as considerably as the close structural resemblance of ovarian epithelial-stromal tumors to some epithelial tumors arising elsewhere within the pelvis and abdominal cavity, may be excused by the shared root (i.e., the primitive coelomic epithelium) of the ovarian surface epithelium and the mesothelium [23]. The two most significant predictive factors for

surface epithelial-stromal tumors are tumor stage and the presence or absence of residual disease after treatment intervention. Histopathological grading of malignant ovarian tumors has caused only modest prognostic application; all the same, the role of molecular markers shows some hope [24].

Recent studies have shown that the traditional parameters used to predict clinical behaviour of EOCs are inadequate for determining chemotherapy response and survival in individual patients. Resistance to chemotherapy, inherent or acquired, precludes long-term survival and cure in the majority of patients. While some patients with advanced stage and bulky disease have complete responses to chemotherapy and achieve cure, other patients with seemingly limited disease can show rapid progression to death. A fuller understanding of tumor biology and factors connected with common resistance should lead to improved handling and survival. In the present work, the authors showed that IGF-1R expression was correlated with OS rates, and that IGF-1R positivity was associated with a number of favorable prognostic factors such as a positive hormone receptor status (ER and PR), absence of HER2 amplification, and a low histological tumor grade. These results might explain the loss of statistical significance in the multivariate analysis of the OS.

The lack of correlation between the genes associated with the epithelial morphology of cancer cell lines and the gene list identified with tumor samples might rest, in character, in the intrinsic limitation of in vitro cultures and in the special features of ovarian surface epithelium. On the single hand, cancer cells stably adapted to growth in vitro tend to lose their dependence cellular environmental signals, so that their gene expression might only partially reflect the in vivo situation.

First line treatment of advanced EOC is generally not modified by operative or histologic factors. Adjuvant chemotherapy is given to all patients with advanced cancer and consists of the combination of platinum and paclitaxel [25, 26].

Chakraborty *et al.* [27], reported that the therapeutic strategies that co-target IGF-1R and HER2 are tested in a HER2-overexpressing breast cancer model. Experiments on the interaction and crosstalk between IGF-1R and HER2 in BT474 cells showed that IGF-1R inhibition leads to a decrease in HER2 phosphorylation. Combining IGF-1R inhibitors with trastuzumab or HER2 kinase inhibitors resulted in a synergistic growth inhibition and increased apoptosis.

In the present work, the role IGF-1R in eliciting EOC was considered using its expression in the cancer afflicted individuals. The trial was double-blinded which enabled the proper statistical evaluation. The IGF-1R immunostaining may be used to sort and select patients who might benefit from IGF-1R-targeted therapy.

In this work, the authors observed positive IGF-1R expression in all ovarian cancer subtypes. Therefore, IGF-1R targeting might represent an attractive strategy for the treatment of ovarian cancer. Drugs that specifically target IGF-R are now being produced. Nevertheless, these strict selection standards, combined with the relative rarity of EOC, resulted in a small sample number of both the transcript profiling and validation experiments. In summary, although making up at least 75% tumor cells, the tissue samples used in the transcript profiling experiments were not micro-dissected and therefore may require a small proportion of stromal elements. Thus, the present results continue to be validated in independent surveys.

Conclusion

In conclusion, the present authors found that positive IGF-1R expression in all ovarian cancer subtypes besides its expression was associated with a favorable prediction. IGF-1R might be a valuable immunohistochemical marker for the prediction of cancer product, and for the choice of patients for IGF-1R targeted therapy.

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Address reprint requests to:
Y.S. LIU, M.D.
Department of Obstetrics and Gynecology
Provincial Hospital
Affiliated to Anhui Medical University
No. 17 Lujiang Road, Hefei
Anhui 230001 (China)
e-mail: liuyusheng365@gmail.com