Loss of IFNγ Receptor Is an Independent Prognostic Factor in Ovarian Cancer

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Abstract

Purpose: There is evidence that IFNγ plays an important role in ovarian cancer development. IFNγ produces numerous antitumor effects and it may be evasion of these effects which allows tumor progression. We postulate that genetic instability in tumor cells may lead to modulation of expression of the IFNγ receptor, thus leading to altered tumor biology and patient prognosis. This hypothesis would support the theory of immunoediting in ovarian cancer.

Experimental Design: Using tissue microarray technology of 339 primary ovarian cancers, the expression of IFNγ receptor was assessed immunohistochemically. Coupled to a comprehensive database of clinicopathologic variables, its effect on these factors was studied.

Results: Tumors expressing high levels of IFNγ receptor had significantly improved survival (P = 0.017) compared with tumors expressing low levels of the receptor; this was also seen with complete receptor loss (P = 0.014). Factors shown to predict prognosis independently of each other were the following: age, International Federation of Gynecologists and Obstetricians stage, and the absence of macroscopic disease after surgery. The level of IFNγ receptor expression and complete receptor loss were independently predictive of prognosis on multivariate analysis. There was no correlation between receptor status and any of the standard clinicopathologic variables.

Conclusions: Loss of IFNγ receptor independently predicts poor prognosis in ovarian cancer. Loss of receptor expression may be responsible for the limited success in the therapeutic use of IFNγ in ovarian cancer trials and highlights a subgroup of high expressing IFNγ receptor tumors which are more likely to be susceptible to such treatments.

Ovarian cancer remains the leading cause of death from gynecologic malignancies and is the fourth most common cause of cancer-related death in women. The 5-year survival rate of ~30% remains essentially unchanged in recent years (1). Although some advances have been made in the development of novel chemotherapeutic agents, the effect on mortality is limited. Recent interest in immunotherapies and other novel treatment modalities aims to provide additional options in ovarian cancer management (2–4). Tumor stage, age, residual disease after surgical debulking, and response to chemotherapy predict prognosis in ovarian cancer; however, there still remain large variations in the behavior of seemingly similar tumors when using these standard clinical variables (5). This discrepancy may be accounted for by variations in tumor phenotype at a molecular level.

There is increasing evidence that the immune system acts as an intrinsic tumor suppressor, preventing tumor development. However, by its very nature, there is variation in tumor cell phenotype associated with DNA microsatellite and chromosomal instability which may facilitate evasion of immune attack. This concept is known as 'immunoediting' and is the mechanism through which genetic instability leads to progressive evasion of the antitumor effects of the immune system (6). Previous work has supported this theory with loss of human leucocyte antigen expression being independently prognostic in both breast and colorectal cancers (7, 8). It is thought that adaptation of the IFNγ pathway occurs during this process (9), specifically there is mounting evidence of a role in ovarian cancer development.

The antitumor effects of IFNγ on ovarian cancer seem to be numerous. Antiproliferative effects have been shown (10, 11) possibly via the down-regulation of the HER-2/*neu* receptor (12) and induction of the class II tumor suppressor gene *H-REV 107-1* (13). Recent results indicate that IFNγ may have direct proapoptotic effects through interaction with the tumor necrosis factor–related apoptosis-inducing ligand pathway (14).

Marth et al. (15) established recently a correlation between expression of IFNγ and improved prognosis, elevation of IFNγ correlating with increased activity of tumor suppressor genes *SOCS-1* and *IRF-1*, and reduced activity of HER-2/*neu*.

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Received 11/29/06; revised 3/10/07; accepted 4/23/07.

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doi:10.1158/1078-0432.CCR-06-2833

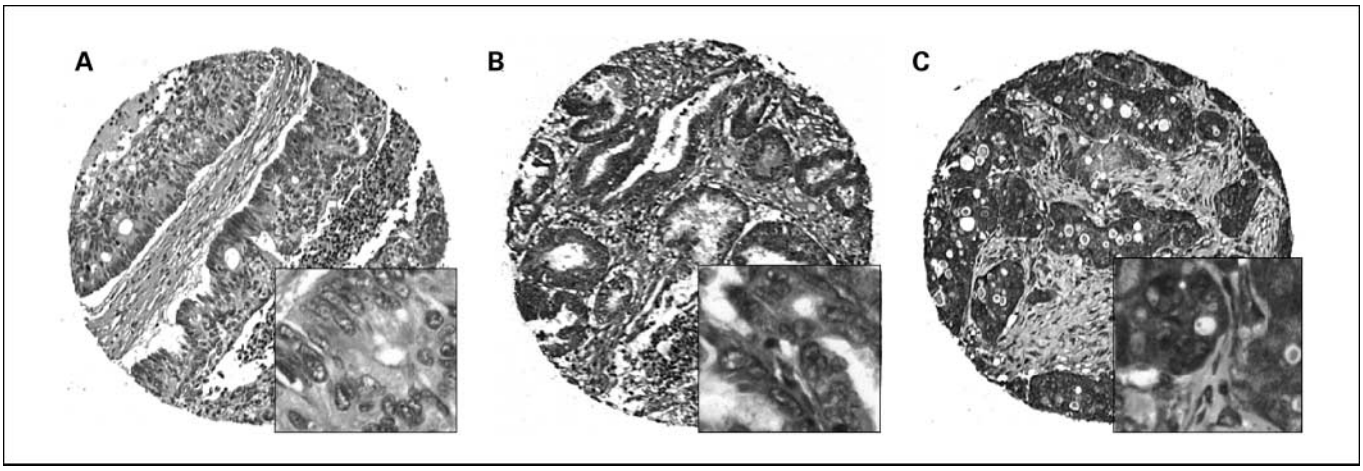

Fig. 1. *A,* a core from tumor showing negative IFNGR1 staining. *B* and *C,* cores of tumor showing moderate (*B*) and strong (*C*) IFNGR1 staining at ×100 magnification. Magnification, ×400 (*inset*).

Studies have shown that, *in vitro*, IFNγ may act synergistically with platinum-based chemotherapy to promote apoptosis and inhibition of proliferation, possibly through the mechanisms described previously (16). The use of IFNγ in a clinical setting is still being evaluated (17), although improvements in progression-free survival have already been seen (18). In light of the accumulating evidence for the antitumor effects of IFNγ, we suggest that ovarian tumors, able to bypass this mechanism, will behave more aggressively and hence result in a poorer prognosis. In keeping with the theory of immunoediting, this evasion may occur via reduced expression of the IFNγ receptor, which has been shown previously to occur in adenocarcinoma cell lines (19).

Materials and Methods

Patients. Patients undergoing laparotomy for primary ovarian cancer at Derby City General Hospital have been followed up prospectively since 1982. Clinicopathologic variables recorded included age, International Federation of Gynecologists and Obstetricians stage, extent of cytoreduction, tumor grade, histologic subtype, details of adjuvant treatment, and disease-specific survival (DSS). DSS was calculated from the operation date until November 31, 2005 when any remaining survivors were censored. The database was audited to ensure validity; there were no major discrepancies with over 97% of data available.

We calculated that a sample size of 300 would allow an 80% chance of detecting a hazard ratio of ≥1.4 and ≤0.7 (EGRET, Cytel Statistical software). Using all cases between January 1, 1982 and December 21, 1997 provided 395 cases for analysis, as well as ensuring that we would have survival data for greater than 5 years for every patient.

During the study period, patients received adjuvant chemotherapy for stages II to IV along with high-grade stage I disease. The type of chemotherapy given varied, but reflected the best current practice at that time; in the latter years, this was mainly platinum based. Sixty-two patients participated in the International Collaborative Group for Ovarian Neoplasia trials I to IV during which the allocated chemotherapy was randomized.

Specimens. All tumors received following resection in the operating theater were incised, fixed immediately in 10% neutral buffered formalin overnight, and then embedded in paraffin wax, ensuring optimal tissue fixation and preservation for histologic examination.

Tissue microarrays (TMA) were constructed as described previously (20). For each tumor, 5-μm section slides stained with H&E were first used to locate representative areas of viable tumor tissue. Needle core biopsies (0.6 mm) from the corresponding areas on the paraffin-embedded tumor blocks were then placed at prespecified coordinates in recipient paraffin array blocks using a manual tissue arrayer (Beecher Instruments). Array blocks were constructed with between 76 to 133 cores in each, and five copies of the array were assembled using different points within the representative tumor area. Fresh 5-μm sections were obtained from each TMA block and placed on coated glass slides to allow the immunohistochemical procedures to be done, preserving maximum tissue antigenicity.

Immunohistochemistry. Immunohistochemical staining for IFNγ receptor expression was done using a routine streptavidin-biotin peroxidase method. Tissue array sections were first deparaffinized with xylene, rehydrated through graded alcohol, and immersed in methanol containing 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase activity. To retrieve antigenicity, sections were immersed in 500 mL of pH 6.0 citrate buffer and heated for 10 min in an 800 W microwave at high power followed by 10 min at low power. Endogenous avidin/biotin binding was blocked using an avidin/biotin blocking kit (Vector Laboratories). To block nonspecific binding of the primary antibody, all sections were then treated with 100 μL normal swine serum for 15 min.

Test sections were incubated with 100 μL mouse anti-human IFNγ receptor 1 (IFNGR1) monoclonal antibody (MMHGR-1, Tebu-Bio), which was found to be optimally diluted at 1:50 (v/v) in TBS for 60 min at room temperature. Positive control tissue comprised whole sections of colorectal cancer. The primary antibody was omitted from the negative control, which was left incubated in normal swine serum.

After washing with TBS, all sections were incubated with 100 μL of biotinylated goat anti-mouse/rabbit immunoglobulin (DAKO Ltd.) diluted 1:100 in normal swine serum for 30 min. Sections were washed again in TBS and next incubated with 100 μL of preformed streptavidin-biotin/horseradish peroxidase complex (DAKO) for 60 min at room temperature. Subsequently, visualization of IFNGR1 expression was achieved using 3,3′-diaminobenzidine (DAKO). Finally, sections were lightly counterstained with hematoxylin (DAKO), dehydrated in alcohol, cleared in xylene (Genta Medica), and mounted with distyrene, plasticizer, and xylene (BDH).

Evaluation of IFNGR1. The IFNγ receptor is made up of two subunits, the first IFNGR1 is responsible for ligand binding and signal transduction and the second IRNGR2 is mainly involved with signal transduction (9). We looked at expression of the former of these subunits as it is most actively involved in IFNγ signaling.

The tumor cores were assessed by two experienced observers (T.J.D. and P.R.); we used two different copies of the TMA providing two samples from different areas of the tumor in each case. The cores were scored independently by the observers with a consensus being reached in difficult cases. Following initial review of the staining characteristics, a semiquantitative system, using both intensity and percentage of cells staining, was adopted as the most accurate method of showing marker expression. The scoring was done in a coded manner with observers blinded to the clinical and pathologic variables of the case. To use both the staining intensity (graded 0, absent; 1, weak; 2, moderate; and 3, strong) and the distribution (percentage cell staining) in the assessment of each core, an intensity score was calculated (percentage cell staining $\times$ intensity of staining); a mean value was calculated for the two cores from each tumor (see Fig. 1).

To determine low and high expression, the median intensity score was used; therefore, an intensity score of >90 defined high IFNGR1 expression.

Statistical analysis. Statistical analysis of the study data was done using the SPSS package (version 13 for Windows, SPSS, Inc.). Pearson χ^2 tests were used to determine the significance of associations between categorical variables. DSS calculations included all patients whose death was related to ovarian cancer. Survival variations between groups were investigated using Kaplan-Meier curves; the log-rank test was being used to estimate sizes of differences in survival. The Cox's proportional hazards model was used for multivariate analysis to determine the relative risk and independent significance of individual factors. In all cases, P values <0.05 were considered as statistically significant.

Ethical approval to carry out the study was granted by the Derbyshire Local Research Ethics Committee.

Results

Of the 395 patients identified within the series, 339 had archived tissue available; these tumors were included in the array. Five of the 339 tumor cores had no tissue available for analysis due to loss during the immunohistochemical processing, leaving over 98% of cases available for analysis; this core loss rate compares favorably with previous TMA studies (see Table 1).

Clinicopathologic characteristics. The median age at diagnosis was 62 years, with only a single case presenting below the age of 30 years. The majority of cases were advanced (n = 206, 63.2%), with serous, endometrioid, and mucinous being the most common subtypes. Over 40% of cases were deemed to be optimally debulked.

This cohort of patients was treated between 1982 and 1997 during which time chemotherapy treatments evolved. The majority of patients received chemotherapy (239 of 339,

Table 1. IFNGR1 expression in all cases included in final analysis including univariate analysis by χ^2 of IFNGR1 status and clinicopathologic criteria

Variable	Analyzed cases, n = 334 (%)	High IFNGR1 expression (%)	Low IFNGR1 expression (%)	χ^2 (P)
Age	n = 333	n = 171	n = 162	1.17 (0.557)
<30	1 (0.3)	0	1 (0.6)	
30-59	133 (39.9)	67 (39.2)	66 (40.7)	
≥60	199 (59.8)	104 (60.8)	95 (58.6)	
FIGO stage	n = 326	n = 168	n = 158	1.86 (0.601)
I	83 (26.5)	41 (24.4)	42 (26.6)	
II	37 (11.3)	18 (10.7)	19 (12.0)	
III	167 (51.2)	85 (50.6)	82 (51.9)	
IV	39 (12.0)	24 (14.3)	15 (9.5)	
Optimal debulking	n = 322	n = 168	n = 157	0.42 (0.838)
Yes	129 (40.1)	67 (40.6)	62 (39.5)	
No	193 (59.9)	98 (59.4)	95 (60.5)	
Tumor grade	n = 332	n = 165	n = 161	0.31 (0.856)
1	38 (11.4)	20 (11.7)	18 (11.2)	
2	72 (21.7)	35 (20.5)	37 (23.0)	
3	222 (66.9)	116 (67.8)	106 (65.8)	
Histologic type	n = 334	n = 171	n = 163	14.53 (0.104)
Serous	177 (53.0)	92 (53.8)	85 (52.2)	
Endometrioid	42 (12.6)	22 (12.9)	20 (12.3)	
Mucinous	34 (10.2)	14 (8.2)	20 (12.3)	
Undifferentiated	51 (15.3)	33 (19.3)	18 (11.0)	
Clear cell	25 (7.5)	8 (4.7)	17 (10.4)	
Other	5 (1.5)	2 (1.2)	3 (1.8)	
Chemotherapy	n = 328	n = 169	n = 163	2.43 (0.487)
Platinum	164 (50.0)	88 (52.1)	76 (47.8)	
Nonplatinum	93 (28.4)	46 (27.3)	43 (27.0)	
Other	71 (21.6)	35 (20.7)	36 (22.6)	
Randomized treatment	57/334 (17.1)	29/171 (17.0)	28/163 (17.1)	0.26 (0.873)
Five-year survival	n = 329	n = 169	n = 160	7.69 (0.006)
Yes	82 (24.9)	53 (31.4)	29 (18.1)	
No	247 (75.1)	116 (68.6)	131 (81.9)	
Alive at time of data censoring	n = 331	n = 170	n = 161	4.508 (0.034)
Yes	56 (16.9)	36 (21.2)	20 (12.4)	
No	275 (83.1)	134 (78.8)	141 (87.6)	

Abbreviation: FIGO, International Federation of Gynecologists and Obstetricians.

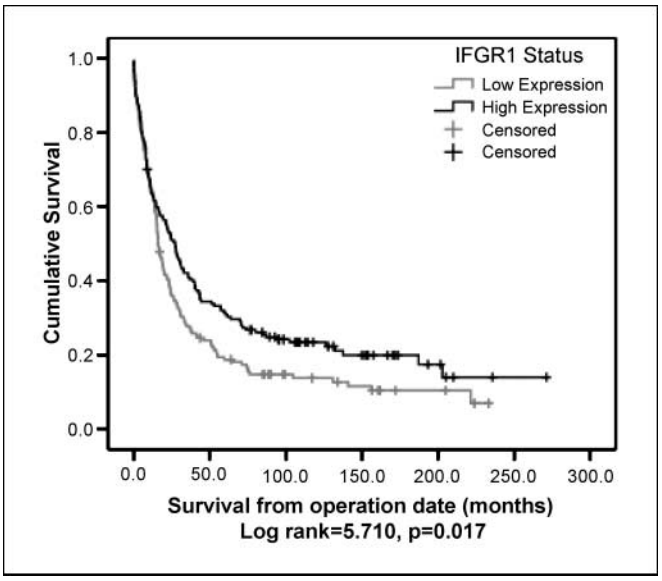

Fig. 2. Kaplan-Meier plot for DSS, high IFNGR1 expression versus low IFNGR1 expression tumors (n = 334).

71%). Carboplatin alone was the most commonly used with 66% (158 of 239) receiving this regime, and combined carboplatin and Taxol given in 4% (9 of 239) and 30% (72 of 239) had nonplatinum-based treatments. The median survival time in the larger groups of platinum and nonplatinum were similar being 21.1 and 18.9 months, respectively. Those receiving combined platinum and Taxol seemed to survive longer (median, 41.8 months); however, this was not significant due to the low number of cases (9). As expected, there was no correlation between IFNGR status and the type of chemotherapy received, with similar numbers receiving the various chemotherapy regimes in IFNGR1-positive and IFNGR1-negative groups. Despite the expected variation in chemotherapy regimes from our study spanning 15 years, there were no significant differences in survival times between the regimes; therefore, this was unlikely to produce confounding effects.

IFNGR1 staining. Staining was seen predominantly within the cytoplasm and cell membrane. Complete lack of staining was observed in 22% of cores; there was an even distribution of staining intensity seen within the remainder with mild, moderate, and strong staining occurring in 23%, 29%, and 26%, respectively. Immunoreactivity in >75% of viable tumor cells was detected in almost half the samples (45%). There was no staining seen within the negative controls.

Using an intensity score allowed the heterogeneity of IFNGR1 expression to be taken into account, with the mean intensity score taken between the two cores from each tumor. The median intensity score of 90 was used to divide the tumors into high and low expression of IFNGR1; using this system, there were 163 low and 171 high cores.

Comparison of IFNGR1 expression and patient tumor characteristics including survival. In a univariate analysis, using the χ^2 test, no significant relationship between IFNGR1 expression and the standard clinical and pathologic variables was apparent. There seemed to be a significant difference in the 5-year survival and the overall survival of patients according to IFNGR1 expression, patients with tumors expressing high levels of IFNGR1 having greater overall and 5-year survival rates (see Table 1).

Correlations between IFNGR1 expression and patient survival were also assessed using Kaplan-Meier survival curves and log-rank testing (see Fig. 2). There was a statistically significant difference in the DSS between patients with high and low IFNGR1 expression, with high expression predicting an improved outcome (P = 0.017; log-rank test, 5.710). The mean DSS was 71 and 46 months for high and low receptor expression, respectively; this relates to a 25-month difference in survival between the two groups.

Although initial classification into high and low expression using the median intensity score was used, a further substratification was analyzed, which divided tumors into three groups,

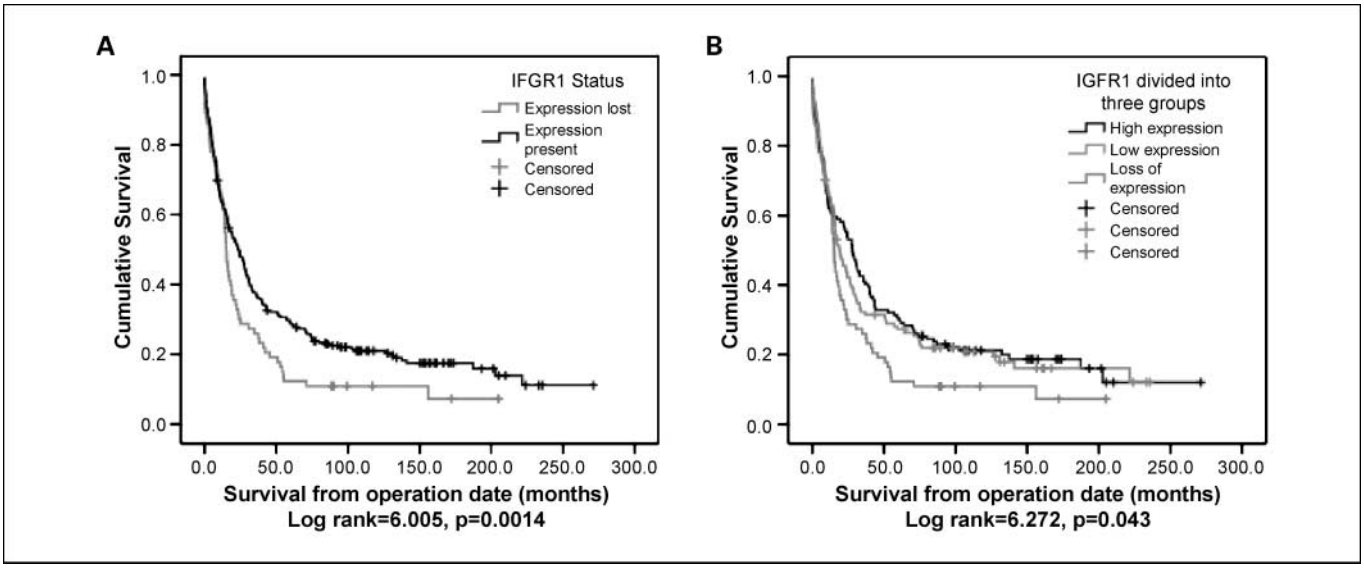

Fig. 3. Kaplan-Meier plots for DSS, IFNGR1 expression, (*A*) present versus loss and (*B*) high versus low versus loss in tumors (n = 334).

those with complete loss of IFNGR1 forming the third group. This showed that much of the difference in survival rates between the patients seemed to be related to complete loss of IFNGR1 (see Fig. 3). Hence, the groups were divided according to the loss or presence of IFNGR1 showing that complete loss was associated with a poorer outcome (P = 0.014; log-rank test, 6.005).

Tumor stage predominantly dictates prognosis in ovarian cancer. Although the effects of IFNGR1 status on patient survival are independent of stage (see multivariate analysis), an analysis of early-stage with late-stage tumors was done to assess if these effects were more marked in one subgroup. Tumors were assessed according to both high/low expression and loss/presence of IFNGR1 (see Fig. 4). Both classifications of IFNGR1 status showed that although the negative effects on patient survival were seen in both early and late stage, the effects may be more pronounced in the early-stage disease (P = 0.042 versus P = 0.053 and P = 0.017 versus P = 0.045).

Multivariate analysis. To assess whether IFNGR1 status was an independent marker of prognosis, the relative influence of IFNGR1 expression and other known standard clinicopathologic prognostic variables were included in a multivariate analysis. Factors shown to predict prognosis independently of each other were age, International Federation of Gynecologists and Obstetricians stage, the absence of macroscopic disease after surgery, and whether the patient received chemotherapy.

These factors along with IFNGR1 status were included in the Cox's regression analysis. IFNGR1 when divided into high and low expression was seen to retain its power to predict an improved prognosis in the study population, independent of other prognostic factors (hazard ratio, 0.727; 95% confidence interval, 0.570-0.927; P = 0.010). This was also seen when patients were divided according to complete loss of IFNGR1 (hazard ratio, 0.744; 95% confidence interval, 0.558-0.992; P = 0.045; see Table 2).

Discussion

Previous authors have established a protective role for IFNγ in ovarian cancer biology (reviewed in ref. 9), although the mechanism through which ovarian cancer is able to evade such action is unclear. In the current study, we investigated a potential route by which the antitumor effects of IFNγ may be avoided, by the down-regulation of IFNγ receptors on the tumor cell surface.

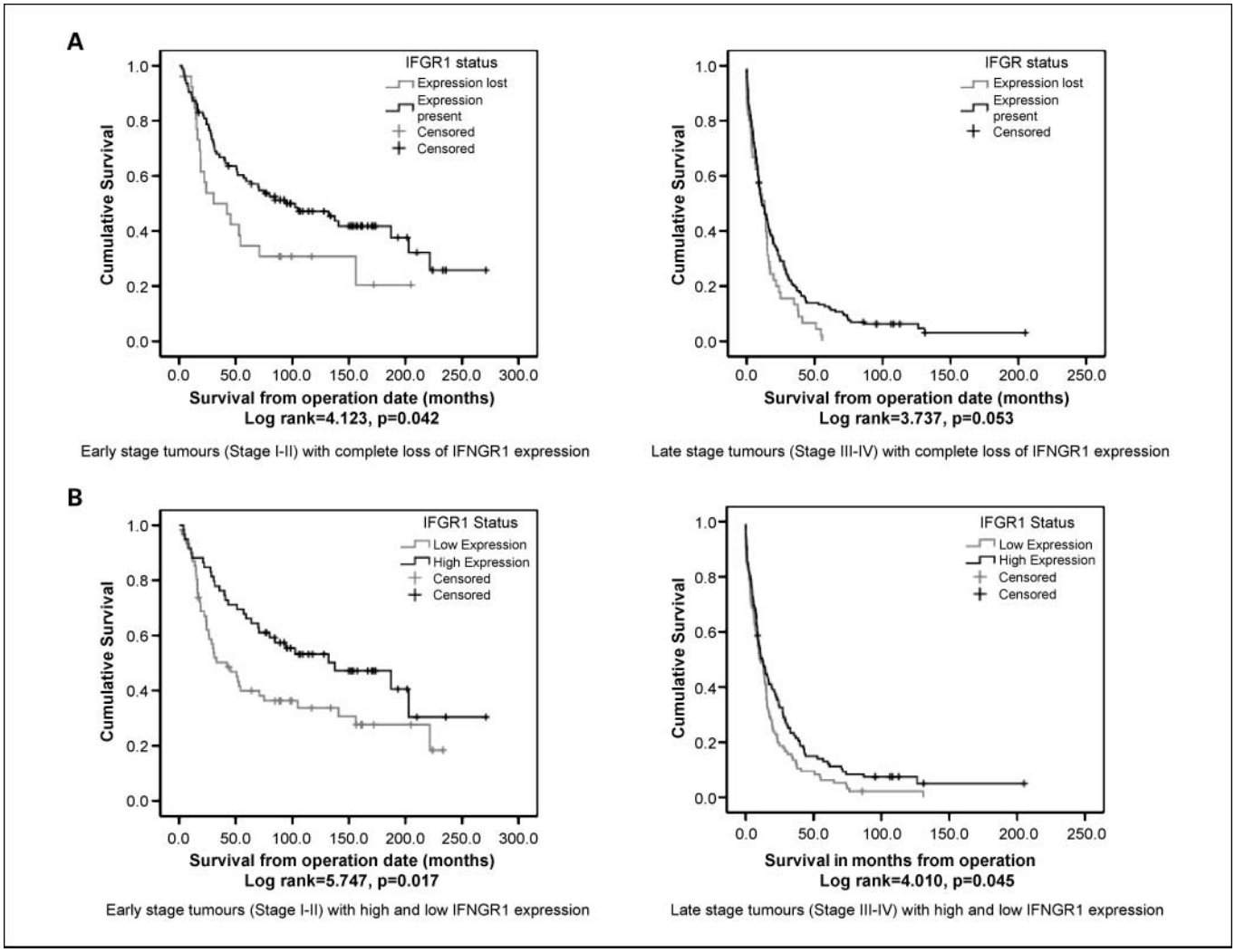

Fig. 4. Kaplan-Meier plots for DSS with early- and late-stage disease using both loss versus presence (*A*) and high versus low (*B*) IFNGR1 expression (n = 334).

Table 2. Multivariate analysis using Cox's regression model showing IFNGR1 expression (low versus high or loss versus present) predicts a positive survival advantage independently of other accepted independent prognostic factors

Variable	HR (95% CI)	*P*
Age at diagnosis (y)	1.024 (1.013-1.036)	<0.001
FIGO stage		
I	1	
II	2.542 (1.469-4.440)	
III	5.091 (3.109-8.330)	
IV	6.851 (3.854-12.180)	<0.001
Optimal debulking		
Yes	1	
No	1.955 (1.389-2.720)	<0.001
Patient received chemotherapy		
No	1	
Yes	0.438 (0.312-0.615)	<0.001
IFNGR1 expression		
Low	1	
High	0.727 (0.570-0.927)	0.010
IFNGR1 expression		
Lost	1	
Present	0.744 (0.558-0.992)	0.045

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

Since its first description in 1998, TMA technology has been used for the immunohistochemical analysis of target protein expression in a wide range of primary tumor types (20). Initial fears that the reduced amount of individual tumor tissue analyzed using this technique might not be representative of the tumor as a whole seems largely unfounded (21). The strengths of this approach lie in its ability to provide a rapid turnover of results from very large patient cohorts while reducing variability in experimental conditions and reducing costs (22). The value of TMA technology is highlighted in this current study of 339 ovarian cancers. With the use of a comprehensive database of clinicopathologic variables, this provided a powerful tool with which to examine the degree of loss of IFNGR1 in ovarian cancer and the effect this had on patient survival. As with all TMA work, we experienced some loss of tissue cores during antigen retrieval; however, this amounted to <2% and therefore had limited effect on the results.

The well-established prognostic markers of tumor stage and degree of tumor debulking at initial laparotomy both proved significant in the study population. Interestingly, if the patient was deemed suitable for and received chemotherapy, there seemed to be a survival advantage; this has not been widely reported and may simply reflect a poor performance status in those patients in whom chemotherapy was deemed inappropriate. Any potentially confounding effects of this finding were avoided by inclusion of this factor in multivariate analysis.

There was variation in both the intensity of staining and the proportion of tumor cells displaying immunoreactivity to IFNGR1 antibody. Using an intensity score, it was possible to incorporate both factors when calculating the degree of receptor expression.

No expression of IFNGR1 was observed in 22% of tumors with only 54% showing moderate to strong staining. Using the median intensity score enabled a statistically valid division into high and low expression of IFNGR1. However, most of the experimental data to date have assessed the potential effects of IFNGR1 loss, as opposed to high and low expression, on tumor growth. As such, a reanalysis was done separating those tumors with complete receptor loss from those any receptor expression. This showed that much of the effects on survival were due to loss, as opposed to simple reduction, of IFNGR1 expression ($P = 0.014$). Using complete loss of IFNGR1 as opposed to variations in degrees of receptor expression may be more biologically valid and perhaps more reliably predict which tumors have functioning IFNγ pathways. The improved DSS in tumors maintaining IFNGR1 suggests that interruption of the IFNγ pathway, and hence evasion of subsequent antitumor effects, leads to a tumor being more aggressive and a poorer prognosis for the patient. This uncoupling from the immune system provides support to the theory of immunoediting in ovarian cancer.

The lack of correlation with any of the histopathologic variables suggests that the effects of IFNGR1 on survival are independent of any associated confounding factors. This is supported by multivariate analysis in which the prognostic value of IFNGR1 expression maintained significance when all other prognostic markers (tumor stage, degree of cytoreductive surgery, and chemotherapy) are included.

Genetic instability leads to progressive dedifferentiation and invasion of tumors. It also creates a wide range of tumor phenotypes which allow evasion of the immune system. However, there is little evidence to suggest that these events are linked. Indeed, in our study, loss of IFNGR1 was not associated with grade or stage but did predict poor survival.

Interestingly, although the effects of IFNGR1 expression on survival were seen at all tumor stages, they may be more pronounced in early-stage disease. This would support the hypothesis that loss of IFNGR1 is an early phenomenon in ovarian cancer development, with these effects being diluted by the further subsequent cellular mutations which occur as the tumor escapes from immunologic control.

The immunoediting theory would suggest that low levels of IFNGR1 should confer a survival advantage to those cells, which would become the predominant phenotype leading to loss of IFNγ responsiveness. This hypothesis has been supported by animal studies. Overexpression of a dominant-negative mutant of IFNGR1 produced enhanced tumorogenicity and reduced immunogenicity in a mouse model (23). Furthermore, mice lacking IFNGR1 or STAT1 were 10 to 20 times more likely to develop tumors in response to the carcinogen, methylcholanthrene, than their wild-type counterparts (19).

Tumor transformation results in an up-regulation of stress molecules that are recognized by γδ T cells and natural killer cells with the subsequent release of high levels of IFNγ. Although there is little natural killer infiltration in ovarian cancer, suggesting that these cells play a limited role in immunosurvelliance of this cancer, mice lacking γδ T cells have high levels of spontaneous tumors (24). IFNγ has direct antitumor activity via effects on proliferation (10, 11), induction of tumor suppressor genes (13, 15), and apoptosis (14). The antiproliferative effects are thought to be via the actions of p21 (10) and p27 that inhibit cyclin-dependent kinases (25). The proapoptotic effects of IFNγ are thought to be

due to induction of intrinsic and extrinsic caspase pathways (26), as well as inhibitory effects on antiapoptotic molecules Bcl-2 and Bcl-X_L (27).

There are now several studies supporting a clinical role for IFNγ, probably through effects on apoptosis, seen *in vitro* (10), with a pilot study showing similar effects *in vivo* with concurrent reduction in ascites production in two patients (28). In a randomized phase III study of 148 women treated with cisplatin and cyclophosphamide as first-line chemotherapy, approximately half received additional IFNγ during each treatment cycle. A significant increase in progression-free survival was observed but not overall survival (18). Recent chemotherapy regimes, which tend to include paclitaxel, seem to be safe with regards to toxicity when combined with IFNγ (17). The effects on survival were the subject of a large multicenter phase III trial, Gamma Interferon and Chemotherapy Efficacy Study, which was recently discontinued due to unfavorable results in the IFNγ plus standard chemotherapy group; however, assessment of the IFGR status of the tumors was not included (29). The illustration from our data that there is loss of IFNGR1 in more aggressive tumors may reduce or even completely negate any potential benefits of IFNγ treatment and hence might explain the lack of effect on overall survival in an unselected population as seen in the Gamma Interferon and Chemotherapy Efficacy Study. IFNγ may prove more effective if targeted at those with an intact IFNγ signaling pathway.

In summary, the expression of IFNGR1 in a typical ovarian cancer population is variable with 22% showing complete loss of the receptor. Reduced receptor expression seems to have a negative effect on survival and is unrelated to other clinicopathologic variables. The low expression of IFNGR1 is an independent prognostic marker in ovarian cancer. These data suggest that evasion of the IFNγ effects, through reduced receptor expression, results in more aggressive tumors, and support the theory of immunoediting. Future work focusing on the targeting of susceptible tumors (those with functioning receptors) to IFNγ therapy may provide improved outcomes.

Acknowledgments

We thank Sam Crockett, Claire Coveney, and Denise Wingate for their help in updating and auditing the final database and Steve Kyte and Andrea Gooding for their help in identifying the archived tissue blocks and manufacturing new sections.

References

1. Quinn M. Cancer Trends in England and Wales, 1950-1999. London: Office for National Statistics; 2001.
2. Bookman MA. Biological therapy of ovarian cancer: current directions. Semin Oncol 1998;25:381–96.
3. Hwu P, Freedman RS. The immunotherapy of patients with ovarian cancer. J Immunother 2002;25:189–201.
4. Kirby TO, Huh W, Alvarez R. Immunotherapy of ovarian cancer. Expert Opin Biol Ther 2002;2:409–17.
5. Rubin SC, Randall TC, Armstrong KA, Chi DS, Hoskins WJ. Ten-year follow-up of ovarian cancer patients after second-look laparotomy with negative findings. Obstet Gynecol 1999;93:21–4.
6. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol 2004;22:329–60.
7. Madjd Z, Spendlove I, Pinder SE, Ellis IO, Durrant LG. Total loss of MHC class I is an independent indicator of good prognosis in breast cancer. Int J Cancer 2005;117:248–55.
8. Watson NF, Ramage JM, Madjd Z, et al. Immunosurveillance is active in colorectal cancer as downregulation but not complete loss of MHC class I expression correlates with a poor prognosis. Int J Cancer 2006;118:6–10.
9. Ikeda H, Old LJ, Schreiber RD. The roles of IFN γ in protection against tumor development and cancer immunoediting. Cytokine Growth Factor Rev 2002;13:95–109.
10. Burke F, Smith PD, Crompton MR, Upton C, Balkwill FR. Cytotoxic response of ovarian cancer cell lines to IFN-γ is associated with sustained induction of IRF-1 and p21 mRNA. Br J Cancer 1999;80:1236–44.
11. Marth C, Helmberg M, Mayer I, Fuith LC, Daxenbichler G, Dapunt O. Effects of biological response modifiers on ovarian carcinoma cell lines. Anticancer Res 1989;9:461–7.
12. Marth C, Muller-Holzner E, Greiter E, et al. γ-Interferon reduces expression of the protooncogene c-erbB-2 in human ovarian carcinoma cells. Cancer Res 1990;50:7037–41.
13. Sers C, Husmann K, Nazarenko I, et al. The class II tumour suppressor gene H-REV107-1 is a target of interferon-regulatory factor-1 and is involved in IFNγ-induced cell death in human ovarian carcinoma cells. Oncogene 2002;21:2829–39.
14. Meng RD, El-Deiry WS. p53-independent upregulation of KILLER/DR5 TRAIL receptor expression by glucocorticoids and interferon-γ. Exp Cell Res 2001;262:154–69.
15. Marth C, Fiegl H, Zeimet AG, et al. Interferon-γ expression is an independent prognostic factor in ovarian cancer. Am J Obstet Gynecol 2004;191:1598–605.
16. Nehme A, Julia AM, Jozan S, Chevreau C, Bugat R, Canal P. Modulation of cisplatin cytotoxicity by human recombinant interferon-γ in human ovarian cancer cell lines. Eur J Cancer 1994;30A:520–5.
17. Marth C, Windbichler GH, Hausmaninger H, et al. Interferon-γ in combination with carboplatin and paclitaxel as a safe and effective first-line treatment option for advanced ovarian cancer: results of a phase I/II study. Int J Gynecol Cancer 2006;16:1522–8.
18. Windbichler GH, Hausmaninger H, Stummvoll W, et al. Interferon-γ in the first-line therapy of ovarian cancer: a randomized phase III trial. Br J Cancer 2000;82:1138–44.
19. Kaplan DH, Shankaran V, Dighe AS, et al. Demonstration of an interferon γ-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A 1998;95:7556–61.
20. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–7.
21. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Lab Invest 2000;80:1943–9.
22. Torhorst J, Bucher C, Kononen J, et al. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001;159:2249–56.
23. Dighe AS, Richards E, Old LJ, Schreiber RD. Enhanced *in vivo* growth and resistance to rejection of tumor cells expressing dominant negative IFN γ receptors. Immunity 1994;1:447–56.
24. Gao Y, Yang W, Pan M, et al. γδ T cells provide an early source of interferon γ in tumor immunity. J Exp Med 2003;198:433–42.
25. Bali A, O'Brien PM, Edwards LS, Sutherland RL, Hacker NF, Henshall SM. Cyclin D1, p53, and p21Waf1/Cip1 expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. Clin Cancer Res 2004;10:5168–77.
26. Barton C, Davies D, Balkwill F, Burke F. Involvement of both intrinsic and extrinsic pathways in IFN-γ-induced apoptosis that are enhanced with cisplatin. Eur J Cancer 2005;41:1474–86.
27. Ossina NK, Cannas A, Powers VC, et al. Interferon-γ modulates a p53-independent apoptotic pathway and apoptosis-related gene expression. J Biol Chem 1997;272:16351–7.
28. Wall L, Burke F, Barton C, Smyth J, Balkwill F. IFN-γ induces apoptosis in ovarian cancer cells *in vivo* and *in vitro*. Clin Cancer Res 2003;9:2487–96.
29. InterMune. InterMune discontinues ovarian cancer trial: Intermune; Feburary 2006.