Interferon regulatory factor-1 (IRF-1) suppression and derepression during endometrial tumorigenesis and cancer progression

Alexandra Giatromanolaki a,*, Michael I. Koukourakis b, Konstantinos Ritis c, Konstantinos Mimidis c, Efthimios Sivridis a

a Department of Pathology, Democritus University of Thrace, Alexandroupolis, Greece
b Department of Radiotherapy/Oncology, Democritus University of Thrace, Alexandroupolis, Greece
c Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece

Received 20 June 2003; received in revised form 27 November 2003; accepted 8 March 2004

Abstract

Interferon regulatory factor-1 (IRF-1) is a tumor suppressor gene presumed to be involved in the control of cellular proliferation and transformation. Given that the IRF-1 is consistently expressed in the normally cycling endometrium, the question was raised of the possible role of IRF-1 in the genesis of endometrial adenocarcinoma. A series of 25 normal and 86 malignant endometria was investigated using immunohistochemical techniques and the anti-IRF-1 polyclonal antibody c-20. Normal endometrial glands were, indeed, consistently reactive with IRF-1. Excluding the invading tumor front, malignant endometria were deprived of IRF-1 reactivity, as 81 of the 86 cases (94.2%) were negative for this antigen. At the invading tumor front, however, IRF-1 was derepressed in tumor cells in 35% of the cases. This phenomenon was independent of the extent of lymphocytic response, but it was associated with thymidine phosphorylase (TP) expression. Furthermore, TP up-regulation and host’s lymphocytic response in the area were directly associated. IRF-1 derepression by invading tumor cells was associated with poor prognosis, independently of FIGO stage. It is concluded that down-regulation of IRF-1 is a constant finding in endometrial tumorigenesis. However, derepression of IRF-1 may occur in a subset of tumors, and this event is associated with TP up-regulation and aggressive tumor behavior.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: IRF-1; Thymidine phosphorylase; Endometrial adenocarcinoma

1. Introduction

Interferon regulatory factor-1 (IRF-1) is a weak transcriptional activator factor encoded in the 5q31.1 region [1]. With the exception of embryonal cells, the level of IRF-1 in normal tissues is low and most commonly induced by exposure of cells to interferons α, β and γ [2,3], prolactin [4] or retinoic acid [5]. Following nuclear translocation, IRF-1 binds to DNA either as a monomer or a dimer composed of IRF-1 and the IFN consensus sequence binding protein (ICSBP) [6,7].

IRF-1 induces typical interferon functions, including induction of TAP1 proteins and histocompatibility antigens, induction of caspas, nitric oxide (iNOS), cyclooxygenase-2 and other molecules [8–11]. These functions converge upon initiating cellular anti-viral and anti-bacterial responses, inflammation and apoptosis. An important function of IRF-1 is the induction of the p21 protein, downstream the p53 tumor suppressor gene, causing G1-cell cycle arrest [12–14]. This growth suppression function of IRF-1 is further supported by studies showing that IRF-1 induces lysyl oxidase and indoleamin deoxygenase that down-regulate the biosynthetic activity of tumor cells [15,16].

Further studies indicated that IRF-1 has tumor suppressor activities given that IRF-1 prevents growth of cells transformed by c-myc and c-foc oncogenes [17],

* Corresponding author. Tel.: +30-25510-75118; fax: +30-25510-74623.
E-mail address: targ@her.forthnet.gr (A. Giatromanolaki).
and reverses the malignant phenotype of transformed tumor cell lines [18]. Besides, inactivation of IRF-1 (i.e. abnormalities in the 5q31.1 chromosome region or splicing aberrations of the IRF-1 mRNA) is linked with leukemia and proleukemia myelodysplastic syndromes [19,20]. Despite these findings, the IRF-1 mediated tumor suppressive activity remains poorly understood, since the loss of IRF-1 is not associated with increased rates of spontaneous tumor development, suggesting that IRF-1 is a tumor susceptibility gene that antagonizes tumor development, but IRF-1 abnormalities may not promote tumorigenesis in the absence of other critical genetic events [21].

In this study we investigated the expression of IRF-1 in human endometrial adenocarcinomas. We provide evidence that although IRF-1 is, by and large, suppressed in these neoplasms, it may be up-regulated in a small subset of cases at the invading tumor front, and that such an event parallels the overexpression at this site of thymidine phosphorylase (TP or PD-ECGF), another IFN-α regulated protein involved in angiogenesis and DNA synthesis.

2. Materials and methods

Formalin fixed, paraffin embedded specimens from 86 patients with endometrial adenocarcinoma, of the endometrioid cell type, retrieved from the files of the Department of Pathology, Democritus University of Thrace, Alexandroupolis, Greece. Twenty-five specimens of normal endometrium (various phases of the menstrual cycle) were also retrieved. All cancer patients had been treated surgically with total abdominal hysterectomy and bilateral salpingo-oophorectomy. Histological typing and grading of the endometrial tumors (grade 1 vs. grades 2 and 3) and the depth of myometrial invasion (<50% vs. >50%) were assessed on hematoxylin–eosin sections, using standard criteria. Lymphatic-vascular space invasion was recorded as being present if tumor cells were seen within a space with a definite and clearly identifiable endothelial cell lining. The follow up of the patients in the series ranged from 5 to 182 months with a median of 81 months.

2.1. Assessment of IRF-1 expression

The IRF-1 expression was assessed immunohistochemically with the IRF-1 c-20 rabbit anti-peptide (sc-497p) polyclonal IgG antibody, recognizing the carboxy-terminus of the human IRF-1. Its specificity was validated with Western Blot analysis in Jurkat whole cell lysate, while c-20 antibody was shown not to react with IRF-2 (Santa Cruz Biotechnology, Santa Cruz, CA). The streptavidin–biotin peroxidase method was applied. Three μm sections were deparaffinized and peroxidase was quenched with methanol and 3% H2O2 for 15 min. Microwaving for antigen retrieval was used (3×4 min). The primary antibodies were applied for 90 min. Following washing with TBS, sections were incubated with a secondary anti-rabbit, anti-mouse antibody (Kwik Biotinylated Secondary, 0.69A Shandon–Upshaw) for 15 min and washed in TBS. Kwik streptavidin peroxidase reagent (039A Shandon–Upshaw) was applied for 15 min and sections were again washed in TBS. The color was developed by 15 min incubation with DAB solution and sections were weakly counterstained with hematoxylin. Normal mouse immunoglobulin-G was substituted for primary antibody as the negative control.

The scoring of tissue sections for IRF-1 expression in tumor cells and tumor-associated stroma was decided according to the patterns of IRF-1 expression revealed by immunohistochemistry, as no previous experience existed with IRF-1 expression in human tumors.

2.2. Assessment of thymidine phosphorylase expression

TP expression was assessed with the P-GF.44C monoclonal antibody [22] using the alkaline phosphatase anti-alkaline phosphatase method as previously described [23,24]. The pattern of TP staining tumor cells is mixed cytoplasmic/nuclear and is located predominantly at the invading tumor front, as previously reported. The percentage of cells with strong cytoplasmic and/or nuclear TP reactivity was recorded in all ×200 optical fields at the invading tumor front. The median value was used as a cut-off point to define cases with low vs. high cancer cell TP reactivity.

2.3. Assessment of lymphocytic infiltration (LI)

The assessment of LI was performed on hematoxylin–eosin sections. The degree of LI response at the invading tumor front was scored as high (if a dense lymphocytic presence was evident in the stroma of more than 50% of the ×200 optical fields used to scan the area) vs. weak (scarce presence of lymphocytes at the invading tumor front or focal lymphocytic reaction in less than 50% of optical fields).

2.4. Statistical analysis

Statistical analysis and graphs were performed using the Instat 3.1 Package and GraphPad Prism 2.01 package (GraphPad software Inc., USA). A Fisher’s exact test or the unpaired two-tailed t-test was used for testing relationships between categorical tumor variables, as appropriate. Survival curves were plotted using the method of Kaplan and Meier, and the log-rank test was used to determine statistical differences between life tables. The end-points were the overall survival from the...
day of surgery. A Cox proportional hazard model was used to assess the effect of tumor variables on overall survival. A \( p \)-value < 0.05 was considered for significance.

### 3. Results and discussion

Interferon regulatory factor-1 (IRF-1), as a tumor suppressor gene, is involved in the control of cellular proliferation and transformation [25]. With regard to the human endometrium, experimental studies indicated that IRF-1 is expressed cyclically throughout the normal menstrual cycle [26], an event regulated by prolactin [27]. Indeed, in our study normal endometrial glands exhibited consistently a strong cytoplasmic reactivity for IRF-1, whether these were in the proliferative (Fig. 1a) or the secretory phase (Fig. 1b) of the menstrual cycle. A weak reactivity was also detected in the stroma surrounding the normal glands. Whether IRF-1 suppression in the endometrium promotes malignant transformation remains obscure, although such a link has been demonstrated in human leukemias [19,20]. Doherty et al. also found IRF-1 expression in ductal carcinomas in situ and in high-grade lymph node positive breast carcinomas [28].

Excluding the invading tumor front, endometrial adenocarcinomas were deprived of IRF-1 in 81 out of the 86 cases, (94.2%) studied (Fig. 1c), suggesting that suppression of IRF-1 may be an important factor for endometrial tumorigenesis. Only in a minority of cases (5/86 of the cases, i.e., 5.8%), IRF-1 expression was noted in the inner tumor areas. Stromal fibroblasts, tumor associated lymphocytes and macrophages were persistently negative (Fig. 1e).

In contrast, a varying extent of IRF-1 reactivity was recorded in the cytoplasm of tumor cells at the invading tumor front (Fig. 1d,e). IRF-1 expression by cancer cells was therefore recorded in all optical fields residing in the invading tumor front (areas of interaction between tumor and normal tissue). The median percentage of tumor cells with IRF-1 expression at the invading front was 10% (mean value 19%; range 0–100%; 95% CI 14.1–24.1). In 41/86 (47.7%) cases studied there was no evidence of IRF-1 expression in the tumor cells, and these cases were considered as negative. In 15/86 (17.4 %) IRF-1 expression was noted in less than the mean value, and these cases were considered as bearing low IRF-1 reactivity at the invading tumor front. The remaining cases (30/86, i.e., 34.9%) showed IRF-1 expression in a percentage of cancer cells higher than the mean value at the invading edge, and these cases were considered as having high IRF-1 reactivity. This tendency for “zonal” IRF-1 activation suggests that the loss of IRF-1 expression in endometrial adenocarcinomas is probably the result of microenvironmental influences at the tumor/normal endometrium interface rather than a consequence of genetic alterations. Such
an event is usually suppressed when the invading front becomes inner tumor area, following advancement of the tumor. Analysis of this IRF-1 prevailing zone, showed no association with conventional histological characteristics of the tumor, including stage, histologic grade, stromal invasion or lymph-vascular space penetration (data not shown).

In previous studies, we showed that thymidine phosphorylase (TP), a potent factor for angiogenesis and DNA synthesis, is indeed increased in a small proportion of endometrial carcinomas (approximately 5%) and that such an up-regulation occurs predominantly at the invading tumor front [23]. Although there is no evidence that IRF-1 is involved in TP regulation, IFNs of IRF-1 and TP was also noted ($p < 0.0001$). We, therefore, investigated the expression of IRF-1 at the invading tumor front in relation with that of TP and the presence of lymphocytes.

Using the criteria stated in Section 2, 20/86 (23.6%) cases analyzed exhibited a high degree of lymphocytic infiltration (high LI) at the invading tumor front. Table 1 shows the correlation of IRF-1 and TP expression with LI. A significant association of high LI with high TP expression was noted ($p = 0.03$). A strong co-expression of IRF-1 and TP was also noted ($p < 0.0001$; Table 1). Linear regression analysis of IRF-1 and TP expression at the invading tumor edge showed a significant association between the expression of these two proteins ($p < 0.0001$, $r = 0.47$). This finding suggests that cytokines released by lymphocytes at the invading tumor front may facilitate IRF-1 reactivation in some endometrial adenocarcinomas, and that such an effect is linked with overexpression of TP. It may also suggest that IRF-1 is directly involved in the up-regulation of TP, but this is mere speculation not supported by experimental evidence and, therefore, deserves further investigation.

Survival analysis was performed to assess the impact of IRF-1 derepression in the prognosis of patients. Fig. 2 shows the Kaplan–Meier survival curves stratified for IRF-1 in stage I patients with endometrial adenocarcinoma and multivariate analysis. Survival was considerably unfavorable for patients with IRF-1 expression at the invading tumor front ($p = 0.04$). Stage of disease and IRF-1 expression were proved to be independent prognostic factors. It appears, therefore, that derepression of IRF-1 is a feature of tumor progression in endometrial carcinomas. In these tumors, TP expression at the invading tumor front is a marker of tumor aggressiveness [23,24] and, at the same site, IRF-1 derepression by invading tumor cells is associated with poor prognosis.

It is concluded that down-regulation of IRF-1 is a constant finding in endometrial tumorigenesis. However, derepression of IRF-1 may at times occur, probably as a result of host’s lymphocytic response, and this event is associated with TP up-regulation and aggressive tumor behavior. Further studies are warranted for a better understanding of the tumorigenic effect of IRF-1 suppression and biologic IRF-1/TP interplay in endometrial adenocarcinomas.

### References


### Table 1

**Association of the degree of lymphocytic response with the expression of IRF-1 and TP at the invading tumor front**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lymphocytic response</th>
<th>IRF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>TP</td>
<td>41</td>
<td>7</td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>High</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>IRF-1</td>
<td>Negative</td>
<td>32</td>
</tr>
<tr>
<td>Low</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

### Figure 2

Figure 2 shows the Kaplan–Meier survival curves stratified for IRF-1 expression at the invading tumor front in patients with stage I endometrial cancer. Multivariate analysis in two models (all cases analyzed and in stage I) is also shown.


