Original article

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c-KIT expression and correlation with chemotherapy resistance in ovarian carcinoma: an immunocytochemical study

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Background: Recent studies have shown that several tumours express c-KIT, a growth factor receptor with tyrosine kinase activity; moreover, clinical results have shown the efficacy of the tyrosine kinase inhibitor, STI571, in c-KIT-positive tumours. The aim of this study was to determine the incidence and correlation with chemotherapy resistance of c-KIT expression in advanced serous, low grade of differentiation, ovarian carcinoma.

Patients and methods: We performed an immunohistochemistry analysis of 56 patients with advanced serous ovarian carcinomas using archival paraffin-embedded specimens.

Results: Intense c-KIT immunostaining was observed in 51.7% of cases. c-KIT expression was statistically correlated with progression of disease after first-line chemotherapy ($P = 0.029$).

Conclusions: c-KIT is also expressed in ovarian carcinoma and it is statistically correlated with chemotherapy resistance. This study suggests the necessity for clinical trials confirming the utility of the tyrosine kinase inhibitor, STI571, in ovarian advanced cancer patients with c-KIT overexpression when these patients have shown no clinical response to conventional chemotherapy.

Key words: chemotherapy, c-KIT, ovarian carcinoma, prognosis, STI571

Introduction

Ovarian cancer is the most common gynaecological malignancy causing death in Europe and North America. Its insidious growth pattern is associated with few specific early symptoms and as a result generally leads to diagnosis of advanced stage disease on clinical examination. Approximately 90% of all patients with ovarian carcinoma underwent surgery followed by chemotherapy. Ovarian cancer is a chemosensitive tumour; numerous chemotherapeutic drugs can produce an objective remission in the majority of cases. Moreover, patients with platinum-resistant ovarian cancer that does not respond to initial therapy, or who have disease that recurs after a short treatment-free interval following first-line therapy, pose a particular problem. In these patients alternate therapeutic options are clearly warranted.

STI571 is a small molecule that selectively inhibits the enzymatic activity of several tyrosine kinases, such as the growth factor receptor with tyrosine kinase activity, a product of the c-*kit* gene [1–2]. The physiological ligand is the cytokine stem cell factor (SFC), also called mast cell growth factor or Steel factor [3]. Mutations of c-KIT that cause constitutive activation of the tyrosine kinase function of c-KIT are detectable in most gastrointestinal stromal tumours (GISTs) [4]. Following initial observations of c-KIT expression and its correlation with bad prognosis in GISTs [5] and because c-KIT seems to be also expressed in ovarian carcinoma [6–9], we have evaluated the presence of c-KIT overexpression in advanced ovarian carcinoma for a new therapeutic prospective: a highly targeted and tailored cancer therapy.

The aim of this study was the examination of the presence of c-KIT and its correlation with chemotherapy resistance comparatively on the response to first-line chemotherapy of a series of advanced serous ovarian, low grade of differentiation, carcinoma patients who had undergone the same surgical and chemotherapeutic treatment.

Patients and methods

Case selection

We have studied 56 consecutive cases of International Federation of Gynecology and Obstetrics (FIGO) stage III ovarian serous carcinoma, grade 3, collected from the files of the Department of Human Pathology and Oncology, University of Florence, from 1985 to 1999. The specimens came from 56 patients with known follow-up, who had undergone surgical and chemotherapy treatment at the Department of Gynecology, Perinatology and Reproductive Medicine, University of Florence. Fully informed consent was obtained from all patients prior to surgery, and tumour samples were collected during surgery. All the patients underwent laparotomy for optimal debulking of the gross neoplastic masses with abdominal hysterectomy, bilateral salpingo-oophorectomy, appendectomy and omentectomy with careful examination of all serosal surfaces and biopsies of any suspected lesions. All the patients presented minimal/absent or bulk (>2 cm) residual disease after surgery.

A postoperative treatment was performed in all patients, and it consisted of combined chemotherapy regimens with modified PEC (six cycles of cisplatin

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80 mg/m^2 and cyclophosphamide 800 mg/m^2 alternating with six cycles of cisplatin 80 mg/m^2 and epidoxorubicin 75 mg/m^2) prior to 1997, and after this with combination chemotherapy with six cycles of carboplatin AUC 5 and paclitaxel 175 mg/m^2/3 h.

We have evaluated the clinical response to first-line chemotherapeutic treatment according to World Health Organization (WHO) methods [10]: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The new Response Evaluation Criteria in Solid Tumours (RECIST) guidelines were evaluated with ultrasonography and CT.

We considered c-KIT expression (positive versus negative), measured using immunohistochemical analysis, in ovarian carcinoma in relation to response to first-line chemotherapy.

A treatment-free interval (TFI) of <6 months was established when the disease recurred <6 months after the completion of primary therapy [11–13]. TFI was defined as the interval time from primary treatment to recurrence and/or metastasis. Cause-specific survival was defined as the survival time from primary treatment to death due to the disease.

Tissue specimens and immunohistochemistry

Specimens were obtained by surgical resection in all cases and fixed in 10% formalin before being processed in paraffin. Hematoxylin–eosin stained sections from each histological specimen were reviewed by two pathologists to confirm the histological diagnosis. One representative paraffin block was selected from each case for further study. For immunohistochemical analysis, 3 μm sections were prepared, each section was deparaffinised using xylene and subsequent hydration. The immunohistochemical studies were performed using the streptavidin–biotin–peroxidase method (UltraVision kit; LAB Vision, Fremont, CA) with diaminobenzidine (DAB) as chromogen and Mayer's hematoxylin as nuclear counterstain. The assays were performed entirely on an automated stainer (BenchMark model; Ventana, Tucson, AZ). Negative and positive controls were included with each run. Sections of strongly positive c-KIT GIST were used as positive control. Negative controls were performed by substituting the primary antibody with non-immune mouse serum. Mast cells in the c-KIT-negative cases acted as a positive internal control. The immunohistochemically stained sections were evaluated without knowledge of the clinical outcome of each patient.

Evaluation of immunohistochemical staining

Positive stain appeared as a cytoplasmic and/or membranous stain. The percentage of positive cells and the degree and cellular distribution of the staining were scored. Only cases showing ≥20% moderate to strong membranous and/or cytoplasmic positivity were scored as positive. No background or non-specific staining of tumour stroma or adjacent ovarian parenchyma was encountered other than positivity in mast cells as expected [14].

To assess interobserver variability in the evaluation of c-KIT expression, the evaluations of one author (M.R.R.) were compared with those of one of the other authors (G.L.T.). Initially the slides were evaluated independently, and those graded diversely were subsequently re-evaluated by the two authors together under a discussion microscope.

Statistical analysis

Fisher's exact test was used to analyse the distribution of c-KIT-positive cases according to clinical response to chemotherapy. A value of $P \leq 0.05$ was considered to be statistically significant.

Results

The follow-up period for each patient was until death or ≥5 years after surgery; the median and mean follow-up were 28 and 38.5 months, respectively, with observed values ranging from 11 to 105 months from the time of surgery and first-line adjuvant therapy. Thirty-one patients presented minimal/absent residual disease after surgery and 25 patients presented bulk (>2 cm) residual disease after surgery.

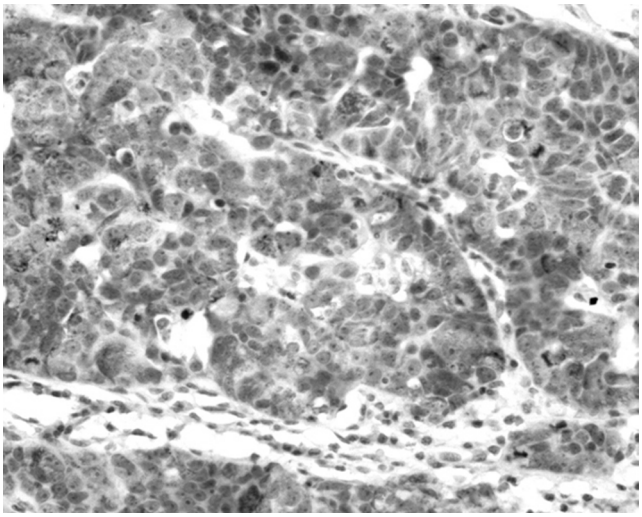

Figure 1. A representative example of a serous ovarian carcinoma with intense c-KIT expression.

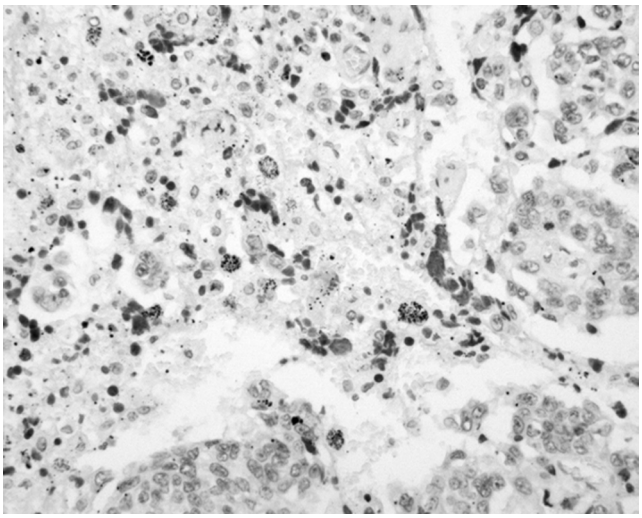

Figure 2. c-KIT-positive internal control: mast cells in a c-KIT-negative ovarian carcinoma.

A complete response to first-line chemotherapy was achieved in 34 patients and a partial response in five patients. None of the patients had stable disease; 17 patients progressed during chemotherapy.

Intense c-KIT immunostaining was observed in the cytoplasm of tumour cells in 29 cases (51.7%). Figure 1 shows a representative example of a serous ovarian carcinoma with intense c-KIT expression. Figure 2 shows mast cells in a c-KIT-negative ovarian carcinoma which act as an internal c-KIT-positive control.

Correlation of c-KIT expression with clinicopathological parameters

c-KIT expression is statistically correlated with progression of disease after first-line chemotherapy ($P = 0.029$; Table 1).

Table 1. Correlation of c-KIT expression with clinical complete response to chemotherapy versus clinical incomplete response to chemotherapy ($P = 0.029$, Fisher's exact test)

c-KIT expression	Clinical complete responsiveness to chemotherapy	Clinical incomplete responsiveness to chemotherapy[a]	*P* value
Positive	14 cases	15 cases	
Negative	21 cases	6 cases	0.029

[a]Partial response or progression.

We observed bulk residual disease following surgery in 15 (51.7%) c-KIT-positive patients and in 10 (37%) c-KIT-negative patients. We also observed a shorter mean TFI and a shorter mean overall survival in c-KIT-positive patients with respect to c-KIT-negative patients. In fact, the mean TFI was 12.7 months in c-KIT-positive cases and 18.4 months in c-KIT-negative cases. The mean overall survival was 31.7 months in c-KIT-positive cases, and 36.8 months in c-KIT-negative cases. c-KIT status was not correlated with residual disease following surgery (minimal/absent versus bulk) nor with clinical outcome.

Discussion

In the current study, c-KIT overexpression detected using immunohistochemistry is observed in approximately half of the ovarian cancers. Thus, the results of this study support the presence of c-KIT in ovarian cultured cells [8], and the hypothesis that c-KIT may also play a critical role in the pathogenesis of ovarian carcinoma [6].

Our results showed that c-KIT-positive cases were less responsive to chemotherapy than c-KIT-negative cases; in fact, c-KIT expression is correlated with progression of disease after first-line chemotherapy. Perhaps c-KIT status may help clinicians identify those patients who would benefit from a different therapeutic approach. Mutation-positive GISTs showed more frequent recurrences and resulted in higher mortality than the mutation-negative GISTs during comparable follow-up periods [5]. Moreover, a more aggressive biological behaviour of c-KIT-positive cases has also been observed in other malignancies, such as small-cell lung cancer [15] and sarcomas [16].

c-KIT is not only a biochemical marker; its involvement in an autocrine, paracrine or endocrine growth loop may represent a molecular mechanism behind aggressive tumour growth [17–18].

The tyrosine kinase inhibitor STI571 has recently attracted much interest [19]. Clinical results obtained with innovative targeted cancer therapies suggest that the major molecular mechanisms that drive tumour growth need to be identified before rational selection of appropriately targeted cancer therapies is possible, based on the specific molecular abnormality in neoplastic cells, such as in chronic myeloid leukaemia [20, 21] and in GISTs [22–23]. c-KIT overexpression in patients with extensive-stage small-cell lung carcinoma [15, 24] and ocular melanoma and liposarcoma [25] may be a potential target for specific immunotherapy with STI571.

Thus, the results of the present study and all these observations provide a rationale for therapeutic intervention aimed at c-KIT expression, particularly in c-KIT-positive ovarian cancer patients with extensive disease; a patient population to whom clinicians currently have little to offer, and for whom new therapeutic strategies are urgently needed.

Therapeutic concepts could include the inhibition of c-KIT with humanized monoclonal antibodies. This approach has already been successfully applied in breast cancer patients using a humanized monoclonal antibody that blocks the extracellular domain of the HER/neu receptor [26].

The current study supports further clinical trials to confirm the utility of the tyrosine kinase inhibitor, STI571, in ovarian advanced cancer patients with c-KIT overexpression, if these patients have shown no clinical response to conventional chemotherapy.

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