Analysis of epidermal growth factor receptor (EGFR) status in endometrial stromal sarcoma

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Summary

Purpose: Endometrial stromal sarcomas (ESSs) are rare neoplasms, which are currently treated by surgery, whereas effective adjuvant therapies have not yet been established. Recently, epidermal growth factor receptor (EGFR) expression has been described in ESS, and a potential role of EGFR-targeted adjuvant therapies has been proposed. The aim of this study was to analyze EGFR status in an ESS series and to evaluate their potential role as molecular targets. *Materials and Methods:* EGFR status was investigated in a total of ten cases of ESS, which included seven low-grade ESS and three undifferentiated ESS cases. EGFR expression levels were assessed by immunohistochemistry, and gene amplification analysis was performed with dual-color fluorescence in situ hybridization (FISH). *Results:* Nine out of ten ESS cases showed positive immunostaining, whereas FISH analysis demonstrated constantly negative results. *Conclusions:* This study confirmed that EGFR is frequently overexpressed in ESS. FISH analysis did not show EGFR amplification in any of the tumors, therefore EGFR expression in ESS should be related to different genetic mechanisms.

Key words: EGFR; Endometrial Stromal Sarcoma (ESS); Immunohistochemistry; FISH.

Introduction

Endometrial stromal sarcomas (ESS) are rare neoplasms, which are currently classified in low-grade (LG-ESS), with indolent growth, tendency to local recurrences and, rarely, to metastasize, and undifferentiated endometrial sarcomas (UES), with ominous prognosis [1, 2].

Histologically, LG-ESS is a well-differentiated neoplasm composed of oval to spindle-shaped cells resembling stromal cells of proliferative endometrium, admixed with numerous small arterioles, similar to the endometrial spiral arterioles. Conversely, UES is defined as a high-grade neoplasm that lacks specific differentiation and shows no histological resemblance to endometrial stroma. Furthermore, UES displays marked nuclear pleomorphism with high mitotic rate and shows destructive myometrial invasion [2, 3].

In LG-ESS, the tumor cells are usually immunoreactive for estrogen and progesterone receptors, CD10, vimentin, and sometimes focally with actin, while they are generally negative for desmin, and h-caldesmon. On the other hand, UES are often estrogen and progesterone receptor-negative [1, 4].

Surgery is still the treatment of choice for ESS. While hormonal therapy has been claimed as a successful therapy to decrease recurrences in LG-ESS, nonetheless, effective adjuvant therapy to prolong survival, either radiation therapy or combination of chemotherapeutic agents, has not yet been established [5-9]. Thus, alternative approaches, such as molecularly targeted therapies, as tyrosine kinases inhibitors, need to be investigated.

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The aim of our study was to analyze epidermal growth factor receptor (EGFR) expression and gene amplification in a series of ESS, to evaluate their potential role as molecular targets.

Materials and Methods

Selection of patients

A series of ten cases of ESS, including seven LG-ESS and three UES cases, was selected from the archives of the Department of Histopathology of the University of Sassari. All the cases were critically reviewed by two experienced pathologists, and categorized according to the current classification.

From formalin-fixed, paraffin-embedded (FFPE) specimens, three micron sections were obtained for haematoxylin and eosin (H&E) stains and immunohistochemical analyses. Consecutive sections were also obtained for fluorescence in situ hybridization (FISH) analysis.

Immunohistochemistry

Immunohistochemistry was performed in serial four µm sections, with the EGFR pharmDx Kit (DakoCytomation, Glostrup, Denmark) according to manufacturer's instructions, as previously described [10]. Antigen retrieval was performed in a proteinase K solution for five minutes. Inactivation of endogenous peroxidase activity was obtained by incubating sections in 3% hydrogen peroxide for five minutes. The slides were incubated with monoclonal mouse anti-human EGFR primary antibody (100 µl) for 30 minutes, and then incubated with labeled polymer HRP (100 µl) for 30 minutes. Immunohistochemical results were evaluated in a semi-quantitative manner and scored according to the intensity of immunostaining (1+, 2+, 3+) and the percentages of positively staining cells. Only cases with more than 1% of immunoreactive cells were considered positive. Membrane and/or cytoplasmic immunoreactivity was also assessed for each positive case.

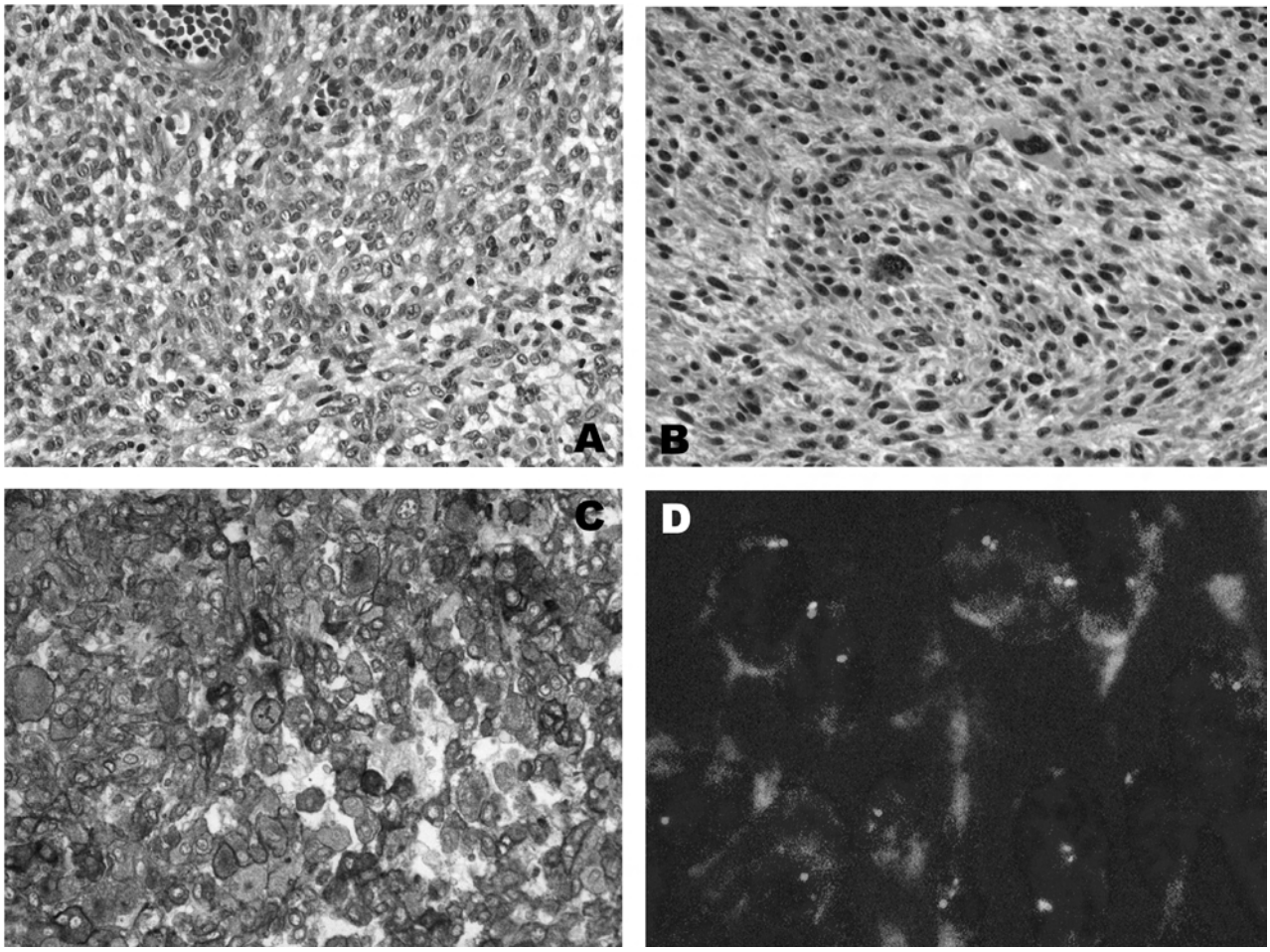

Figure 1. — Morphologic, immunohistochemical, and FISH features. **A)** LG-ESS. H&E stain showing a proliferation of well-differentiated stromal cells, reminiscent of proliferative endometrial stroma (original magnification 400X); **B)** UES. H&E stain with marked cytological atypia, as observed high-grade (original magnification 400X); **C)** UES. Immunostaining for EGFR displaying diffuse and strong, membranous or membranous-cytoplasmic immunoreactivity (original magnification 400X); **D)** FISH analysis on LG-ESS, showing paired orange and green signals, in a case with O/G ratio of about 0.9 (original magnification 1000X).

Fluorescence in situ hybridization

FISH was performed as previously described [11]. Dual-color FISH was performed by using a mixture of a chromosome 7 centromeric region (CEP7 Spectrum Green) DNA probe, and EGFR gene (LSI Spectrum Orange) DNA probe. Probes (5 µl) were added to the slide in a reduced light condition. The slides were covered with a 22 x 22 mm cover slip and sealed with rubber cement. Denaturation was achieved by incubating the slides at 75°C for ten minutes in a humidified box and then hybridized at 37°C overnight. The cover slips were removed and the slides were washed extensively and further air-dried and counterstained with ten ml DAPI (Insitus, Albuquerque, NM, USA). The slides were examined using an Olympus fluorescence microscope with appropriate filters for DAPI, Spectrum Green (CEP7) and Spectrum Orange (EGFR gene). From each tumor section, at least 50 neoplastic nuclei were scored for both orange and green signals, under the fluorescence microscope with x1,000 magnification, and the ratio between orange and green signals was subsequently calculated. Only cases with ratios of two or higher were considered amplified.

Results

Patients' age ranged from 42 to 71 years (mean 52). Eight out of ten cases (five LG-ESS and three UES) occurred in the uterus, whereas the remaining two cases were extra-uterine, arising from foci of pelvic endometriosis, with involvement of large intestine in a single case. Tumor sizes ranged from two to 17 cm (mean seven). All the cases showed immunoreactivity for CD10 at the time of the diagnosis, with positively-stained tumor cells ranging from 10% to 90%. Hormonal receptors expression was reported to be variable, with estrogen receptors (Er) positivity in 60% of the cases, and progesterone receptors (PGr) positivity in 70% of the cases, respectively.

Nine out of ten cases (90%) showed positive immunostaining. Six out of seven LG-ESS cases were positive, showing both membranous and cytoplasmic (five cases), or only membranous staining (one case). The staining intensity was interpreted as 3+ (three cases), or 2+ (two

Table 1. — *Immunohistochemical and FISH results*.

Case	Histology	EGFR Immunohistochemistry Staining Intensity	% of positive cells	EGFR FISH
1	LG-ESS	–	–	Ratio O/G: 1.1
2	LG-ESS	3+	70%	Ratio O/G: 1.05
3	UES	2+	80%	Ratio O/G: 1.1
4	LG-ESS	3+	80%	Ratio O/G: 1.06
5	LG-ESS	2+	60%	Ratio O/G: 0.9
6	LG-ESS	2+	70%	Ratio O/G: 1.1
7	UES	3+	70%	Ratio O/G: 1.3
8	UES	1+	60%	Ratio O/G: 1,1
9	LG-ESS	2+	60%	Ratio O/G: 1.05
10	LG-ESS	3+	70%	Ratio O/G: 0.95

LG-ESS = low-grade endometrial stromal sarcoma; UES = undifferentiated endometrial sarcoma; Ratio O/G = ratio between spectrum orange (EGFR) signals and spectrum green (CEP7) signals.

cases), with percentages of positive cells ranging from 60% to 80%. All three cases of UES were positive for EGFR, with membranous and cytoplasmic (two cases) or only membranous (one case) staining. The staining intensity was evaluated as 1+, 2+ and 3+, with percentages of positive cells ranging from 60% to 80%. No immunoreactivity was recognizable in normal, peritumoral tissues.

FISH analysis showed EGFR/CEP7 ratios constantly below the cut-off value, ranging from 0.9 to 1.3. The results are summarized in Table 1. Figure 1 shows morphologic, immunohistochemical, and FISH features.

Discussion

This study confirmed that EGFR expression is frequently observed in ESS. EGFR immunohistochemical expression in ESS was firstly assessed by Moinfar *et al*. [12] in a series of 23 cases, specifically 20 LG-ESS and three UES cases. EGFR immunoreactivity was appreciable in 17 out of 23 cases (74%), namely 14 out of 20 LG-ESS (70%) and three UES (100%). No genetic analyses were performed in this study. An additional ESS clinical case report by Mitsuhashi *et al*. described focal EGFR immunohistochemical expression in a UES variant, with temporary response to imatinib-mesylate [13]. Recently, Cheng *et al*. performed a comprehensive analysis of targeted tyrosine kinases receptors on 13 LG-ESS by means of immunohistochemistry, describing EGFR negative results in their series [14].

The results in this study confirm and strengthen Moinfar's data, since an immunoreactivity was found in 90% of ESS, specifically 86% of LG-ESS, and 100% of UES.

Genetic analyses on EGFR gene amplification are very scarce. Until now, only Mitsuashi *et al*. reported EGFR immunoreactivity associated with gene amplification, as detected by FISH [13].

In this study, EGFR gene amplification was not identified in all the investigated cases by FISH, hence suggesting that EGFR overexpression in ESS does not involve gene amplification. Therefore, EGFR overexpression should be related to different genetic alterations, or post-translational regulation machinery, with anomalous protein stabilization or defective receptor downregulation increasing its ligand-mediated activation [15, 16].

Clinical significance of EGFR gene abnormalities has been previously stated in non-small-cell lung cancer (NSCLC), and the presence of specific mutations on EGFR gene exons 18-21 has been claimed to determine sensitivity to biologic targeted treatments, such as tyrosine kinase inhibitors gefitinib and erlotinib. Furthermore, concurrent EGFR gene amplification and somatic mutations have been described as increasing neoplastic sensitivity to targeted therapies [17].

In conclusion, in the authors' experience, ESS failed to demonstrate EGFR gene amplification, suggesting that these tumors are likely to be less sensitive to specific tyrosine kinase inhibitors; nevertheless, EGFR protein overexpression, determined by immunohistochemistry, could as well be taken into account as a potential target for anti-EGFR monoclonal-antibody-based therapies.

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