c-kit overexpression in neuroendocrine small cell carcinoma of the uterine cervix

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Summary

Purpose of investigation: Neuroendocrine small cell carcinoma of the uterine cervix (NESCC) grows aggressively, and is resistant to anticancer agents and radiation, having an extremely poor prognosis. The incidence of c-kit proto-oncogene overexpression is high in gastrointestinal stromal tumors (GISTs) and small cell lung cancer, and tyrosine kinase inhibitors have been used effectively to treat GISTs. Few studies have investigated whether c-kit is overexpressed in NESCC. To investigate whether NESCC can be a target for molecular targeted therapy with tyrosine kinase inhibitors, we examined the expression of c-kit in this tumor.

Methods: Twenty-one NESCCs were examined for c-kit expression by immunohistochemical staining using the labeled streptavidin-biotin complex (LSAB) method. The expression of c-kit was regarded as positive (overexpression) and negative when the membrane and cytoplasm of more or less than 25%, respectively, of tumor cells were stained.

Results: Nine NESCCs (43%) were c-kit-positive (overexpression). No difference in age or clinical stage was noted. No difference in prognosis was observed between the c-kit-positive and –negative patients.

Conclusion: The incidence of c-kit overexpression was high in NESCC; therefore, the patients with this tumor may become a future target for molecular-targeted therapy with tyrosine kinase inhibitors.

Key words: c-kit; Neuroendocrine small cell carcinoma; Uterine cervix.

Introduction

Neuroendocrine small cell carcinoma of the uterine cervix (NESCC) is known as a rare disease with a poor prognosis, showing aggressive growth [1-4]. Since it is resistant to anticancer agents and radiation, no appropriate therapy is currently available [1-4].

The c-kit proto-oncogene encodes a transmembrane tyrosine kinase receptor (CD117), and has been reported to be frequently overexpressed in gastrointestinal stromal tumors (GISTs) [5-7], which serves as a criterion for the diagnosis of GISTs [7]. Recent studies have reported that c-kit overexpression is also frequently seen in small cell lung cancer [8-11]. Interestingly, *in vitro* studies have shown that STI571, a tyrosine kinase inhibitor, inhibits the growth of small cell lung cancer cells [12, 13].

To investigate whether NESCC can be a target for molecular-targeted therapy with tyrosine kinase inhibitors, we examined the expression of c-kit in this tumor.

Patients and Methods

Patients

Twenty-one patients with NESCC were studied who had been treated in the period 1983-2003 at the Department of Obstetrics and Gynecology, Jichi Medical School Hospital or Kitasato University Hospital. Their median age was 47 years (ranging from 28 to 65). Eleven patients had clinical Stage I, four had Stage II, four had Stage III, and two had Stage IV disease.

NESCC was diagnosed by hematoxylin-eosin and glimerius staining, and by supplementary immunohistochemical staining for neuroendocrine markers such as chromogranin, synaptophysin, and neuron-specific enolase.

Immunohistochemical staining of c-kit

For the immunohistochemical staining of c-kit, formalin-fixed, paraffin-embedded tissue was cut into 4-μm sections, and stained by the labeled streptavidin-biotin complex (LSAB) method. Briefly, deparaffinized sections were autoclaved in 0.01 M citrate buffer (pH 6.0) at 121°C for 5 min for antigen retrieval. After blocking endogenous peroxidase activity by incubation with 3% hydrogen peroxide at room temperature for 10 min, sections were incubated at 4°C overnight with a 1:50 dilution of specific anti-c-kit mouse monoclonal antibody (CD117, Novocastra Lab. Ltd., Benton Lane, Newcastle upon Tyne, UK), followed by reaction with biotinylated link antibody and peroxidase-labeled streptavidin at room temperature for 10 min each. Finally, the sections were reacted with DAB substrate-chromogen solution, and counterstained with hematoxylin. The LSAB kit used was purchased from Scytek Lab (Logan, Utah, USA). Normal mouse serum was used as a negative control.

The expression of c-kit was regarded as positive (overexpression) and negative when the membrane and cytoplasm of more or less than 25%, respectively, of tumor cells became stained.

Statistical analysis

Statistical analysis was performed using the Fisher's exact probability test and the generalized Wilcoxon test; $p < 0.05$ was considered significant.

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Fig. 1a

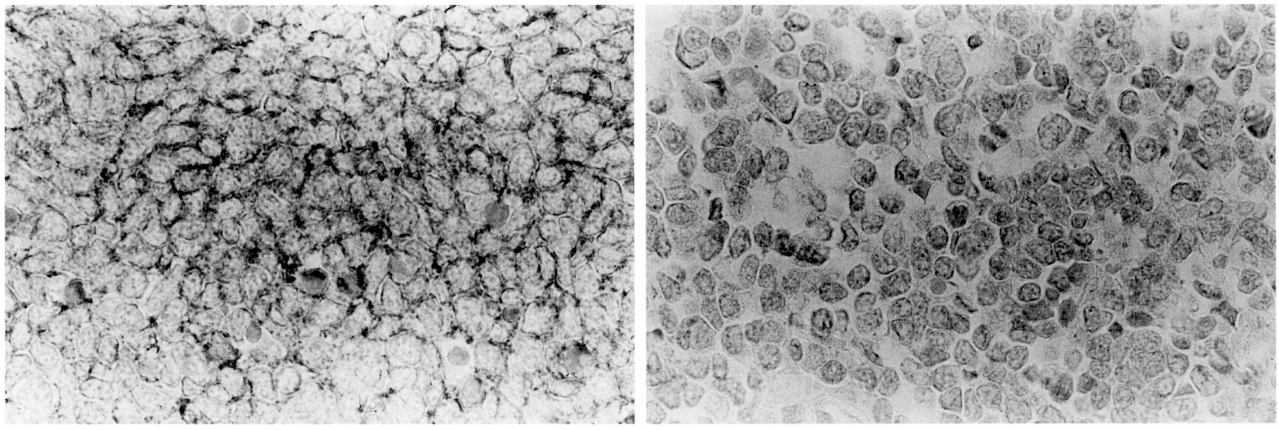

Fig. 1

Figure 1. — c-kit-overexpression of NESCC; a) The membrane and cytoplasm of tumor cells were markedly stained (× 400). b) Negative control (× 400).

Results

Nine of the 21 NESCCs (43%) were judged to be positive for c-kit overexpression (Figure 1A). In three NESCCs, more than 50% of the tumor cells were positively stained.

The incidence of positive staining by age was 44% (7/16) in patients under 50 years of age, and 40% (2/5) in patients 50 years or older, showing no difference between the two age groups. The incidence of positive staining by clinical stage was 55% (6/11) for Stage I disease and 30% (3/10) for Stage II-IV disease, showing no significant difference between the two clinical stage groups (Table 1). Survival curves for the c-kit-positive and –negative groups are drawn in Figure 2, showing no difference: the c-kit-positive and –negative patients had 3-year survival rates of 36% and 25%, respectively.

Table 1. — *Association between age, clinical stage and c-kit expression.*

	c-kit expression		p value
	Positive n (%)	Negative n (%)	
Age			
50 > (n = 16)	7 (44)	9 (56)	0.647
50 ≦ (n = 5)	2 (40)	3 (60)	
Clinical stage			
I (n = 11)	6 (55)	5 (45)	0.245
II-IV (n = 10)	3 (30)	7 (70)	

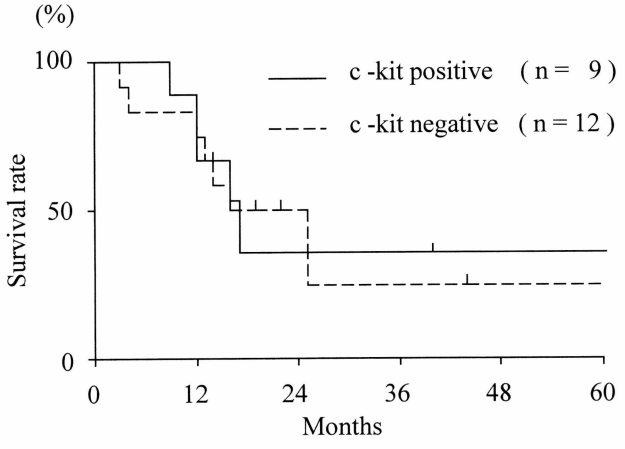

Figure 2. — Association between c-kit expression and survival time in patients with small cell carcinoma of the uterine cervix. Survival curves showed no difference between the c-kit-positive and negative patients.

Discussion

In this study, c-kit overexpression was detected in 43% of NESCCs. Published studies on c-kit expression are very scanty, and only Wang *et al.* [11] reported that the incidence of c-kit overexpression in NESCC was lower, at 27%, than that in this study. This discrepancy may have been due to differences in the specific antibodies used and in reaction conditions, as indicated by Lonardo *et al.* [14]. Wang *et al.* [11] used a specific antibody from DAKO, and performed the incubation at room temperature for one hour, whereas we used an antibody from Novocastra for incubation at 4°C overnight.

No associations were noted between c-kit overexpression and clinical characteristics such as age, clinical stage, and prognosis. Studies have reported that c-kit overexpression in small cell lung cancer is associated with a good prognosis [8] or poor prognosis [10], or unassociated with prognosis [9], providing no consensus.

It has been confirmed that c-kit is overexpressed in almost all patients with GISTs, for which tyrosine kinase inhibitors, such as STI571, are clinically effective [15, 16]. This suggests that c-kit-overexpressing malignant tumors other than GISTs can also be molecular targets of tyrosine kinase inhibitors [17]. For example, c-kit overexpression is frequently observed in small cell lung cancer. Clinical trials of tyrosine kinase inhibitors in this tumor are in progress, and STI571 is reportedly effective in some patients [18]. NESCC closely resembles small cell lung cancer in morphological aspects including ultrastructural features [19]. The present study also showed frequent c-kit overexpression similar to that in small cell lung cancer, suggesting that tyrosine kinase inhibitors may also prove effective for NESCC.

In GISTs, immunohistochemically demonstrated c-kit overexpression is almost always associated with c-kit mutations [20-22], suggesting that c-kit protein overexpression represents c-kit mutations. In other words, we speculate that, in patients with c-kit-overexpressing GISTs, tyrosine kinases are constitutively activated through mutation [17, 23], and can be good targets of tyrosine kinase inhibitors such as STI571. On the other hand, in small cell lung cancer, the relationship between immunohistochemically demonstrated c-kit overexpression and mutation has not been clarified. Also, in NESCC, the relationship between c-kit protein overexpression and mutation has not been elucidated. Therefore, tyrosine kinase inhibitors may not be as effective for small cell lung cancer or NESCC as for GISTs. Although we analyzed one c-kit-overexpressing NESCC for mutations in exons 9, 11, 13, and 17, we were not able to confirm such mutations (data not shown). However, the high incidence of c-kit overexpression in NESCC suggests the possibility that tyrosine kinase inhibitors may target c-kit in this tumor. Molecular targeted therapy with tyrosine kinase inhibitors targeting c-kit may be a promising treatment for NESCC, for which no appropriate therapy is currently available.

Conclusion

The incidence of c-kit overexpression was high in NESCC; therefore, patients with this tumor may become a future target for molecular-targeted therapy with tyrosine kinase inhibitors.

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