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THE EXPRESSION OF THE fms GENE AND THE GENE PRODUCT IN THYROID TUMOR

SATOMI HAGA[1], MASAHIKO INUI[2], HUMIO MATSUZUKA[3], SHOUICHI KATAYAMA[4], HIROSHI YAMAMOTO[1] and YOSHIO HIASA[5]

[1] 2 *nd Department of Anatomy, Nara Medical University,*

[5] 2 *nd Department of Pathology, Nara Medical University*

[2]*Department of Surgery, Daiyu-kai Hospital*

[3]*Department of Surgery, Kuma Hospital*

[4]*Department of Clinical Pathology, National Kure Hospital*

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Summary: The amplification of the fms gene DNA was investigated in 5 human thyroid tumors. No significant amplification or rearrangement was observed in tumor DNA. The expression of the fms gene product was also investigated in 20 thyroid tumor tissues embedded in paraffin using a polyclonal antibody to the fms oncogene product immunohistochemically. Ten out of 20 samples showed clearly positive, 6 out of 20 were weakly positive but 4 out of 20 were negative. Therefore, the fms oncogene might play an important role for thyroid carcinogenesis, and it might also be of possible importance for understanding the mechanism of thyroid carcinogenesis.

Index Terms

human DNA, thyroid tumor, fms gene, fms gene product

INTRODUCTION

The v-fms gene is a viral oncogene found in Susan McDonough-feline sarcoma virus (SM-FeSV) in 1971[1]. The v-fms gene product, which is associated with tyrosine kinase, is formed in the SM-FeSV infected cells with complex processing and finally gp $120^{fms}$and gp $140^{fms}$are produced in the cells[2,3]. This fms proto-oncogene was identified with the receptor of macrophage colony stimulating factor (CSF-1 or M-CSF) gene[4] using immunoprecipitation method. Recently the functions or structures[5] of the fms gene or the gene product have been studied in relation to carcinogenesis. However, no systematic study has been performed so far on the role of the fms gene in human thyroid tumor. We describe fms gene amplification and the expression of the fms gene product in human thyroid tumors.

MATERIALS AND METHODS

1) DNA Preparation: Five thyroid tumors and peripheral blood cells were obtained at the Department of Surgery, Daiyu-kai Hospital and Kuma Hospital. High molecular weight DNA was prepared according to the method of Sambrook et al[6].

2) Digestion of DNA with Restriction Endonuclease and Agarose Gel Electrophoresis: DNA(5 μg) was digested with EcoRI under a condition described by the supplier, electrophoresed on 0.7% agarose gels and transfered to nitrocellulose filters following the procedure of

Southern with minor modification[7].

3) Preparation of Probe and Hybridization: v-fms probe was obtained from Japanese Cancer Research Resources Bank and was labeled with ^{32}P-dCTP by nick translation[8]. The filters were hybridized with the ^{32}P-labeled probe at 65℃ for 24 hr. Filters were washed sequentially in 2 xSSC (SSC; 0.15 M NaCl, 0.015 M Sodium citrate) plus 0.1 % sodium dodecyl sulfate (SDS) for 5 min at room temperature, two times in 0.1 xSSC plus 0.1% SDS for 20 min at 65℃, and in 2 xSSC for 30 min at room temperature.

4) Immunohistochemistry: Samples of 20 paraffin embedded thyroid tumors were obtained from the 1 st Department of Surgery and the Department of Otorhinolaryngology Nara Medical University Hospital. Tissues were sectioned in 2 - 3 μm and immunostaining was performed using the HISTOFINE Kit(Seikagaku Co., Tokyo, Japan) according to the manufacturer's recommendation. Polyclonal antibody to fms oncoprotein was purchased from Cambridge Reseach Biochemicals Inc. (Wilmington, DE, USA).

RESULTS AND DISCUSSION

DNA was extracted from 5 thyroid tumors and 5 peripheral blood cells from the same patients as control. As shown in Fig. 1, EcoRI digestion of tumor DNA and control DNA yield one or two fragments which hybridized with the v-fms probe. Three patients showed only 17 Kb fragment but others showed additional fragment of 23 Kb. As tumor DNA and control DNA showed the same pattern, additional fragment is not due to rearrangement but is due to pleomorphism. In these 5 patients, there was no amplification of fms gene in tumor DNA. Though the amplification or rearrangement of the fms gene may not be needed for thyroid tumorigenesis, the number examined in this study was small, so further study must be performed in this respect.

The results of immunohistological evaluation of thyroid tumor are summarized in Table 1. Of 20 tumors, ten (50%) showed clear positivity in the cytoplasm as well as inside the follicle.

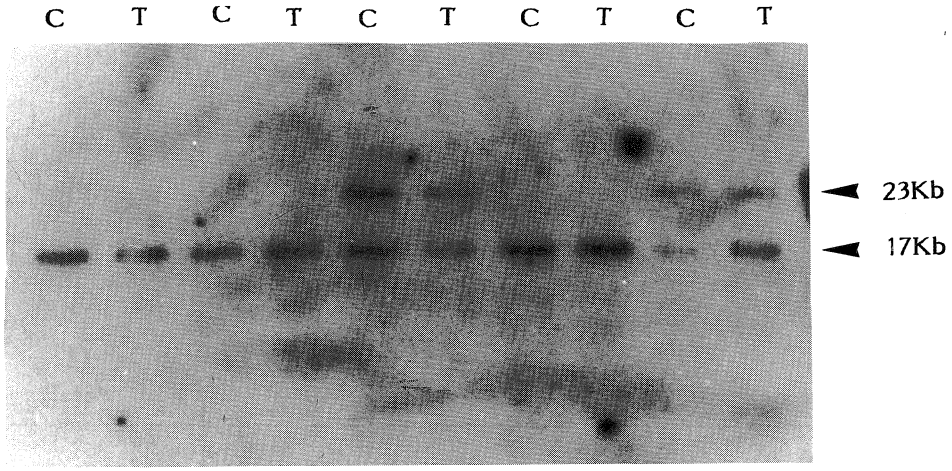

Fig. 1. A southern hybridization pattern of the DNAs from 5 thyroid tumors(T) and peripheral blood cells(C) from the same patients.
Samples were digested with Eco RI and probe used was v-fms.
The positions of 23Kb and 17Kb fragments are noted in the right margin.

Table 1. The expression of the fms gene product in thyroid tumors

Examined number	Positive (%)	Weakly Positive (%)	Negative (%)
20	10 (50)	6 (30)	4 (20)

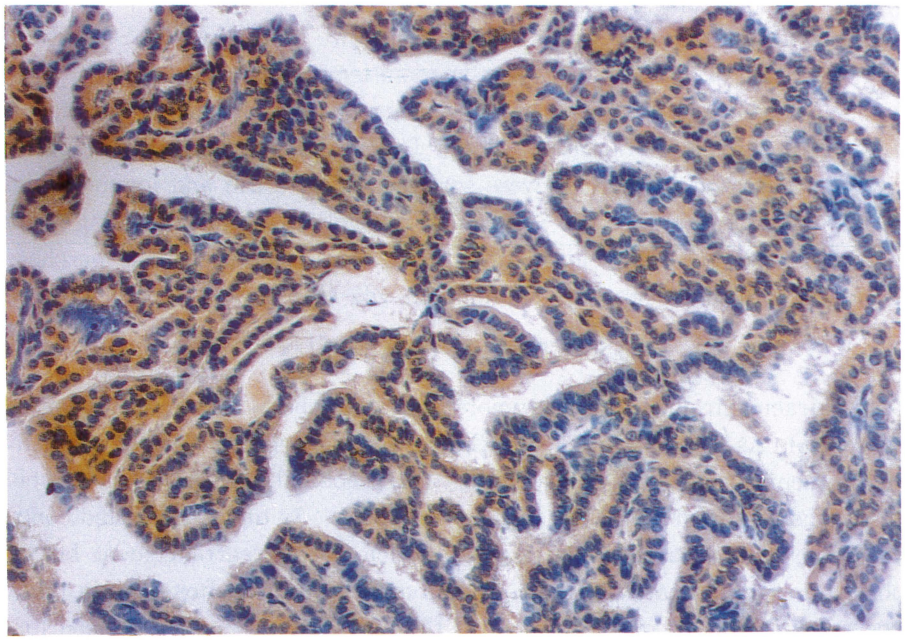

Fig. 2a. A positive staining pattern of the fms gene product using anti fms polyclonal antibody. (×100)

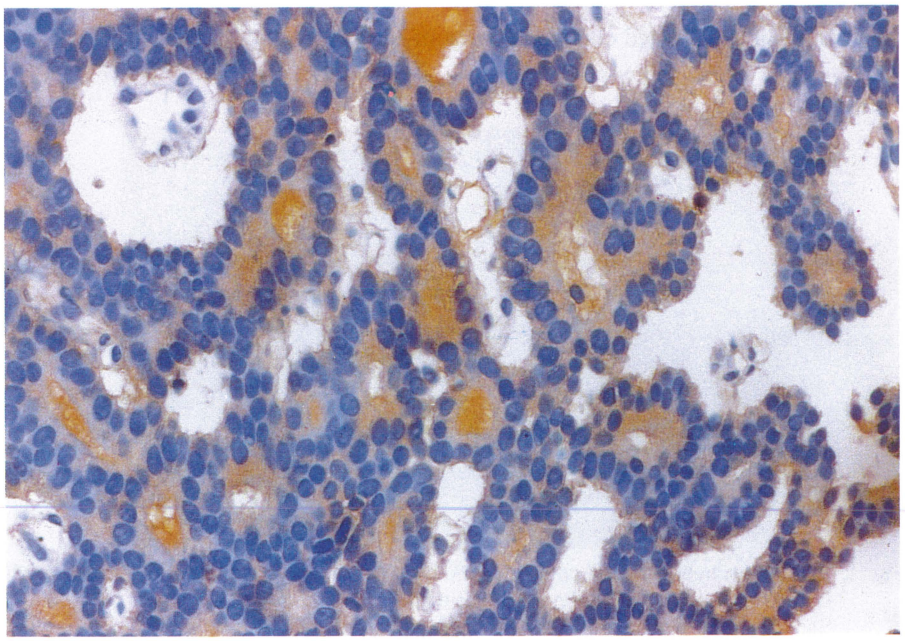

Fig. 2b. Higher magnification of Fig. 2a. (×400)

Six (30%) showed weak positivity and 4 (20%) showed negativity. As shown in Fig. 2, the positive pattern of staining in cytoplasm and inside the follicle were clearly observed using a polyclonal antibody to fms oncoprotein. Though more than 50% samples of paraffined blocks showed positivity or weak positivity, no amplification or rearrangement was seen in Southern hybridization. Therefore, in the DNA level, no amplification or rearrangement of the fms gene has taken place in thyroid tumorigenesis. It is, however, from the results of immunohistochemistry, the fms gene product will be expressed or secreted into follicle.

As more than 50% of the patients showed positivity using anti-fms oncoprotein, fms protein seems to be related with and furthermore seems to play an important role for thyroid tumorigenesis. On the other hand, we still have relatively high incidence of the fms product negative patients. There must be undetermined factors which control thyroid tumorigenesis, which is important to ascertain the differences between fms product positive and negative thyroid tumor patients. Further study must be performed in this respect.

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