Mutation of the SRC Gene in Endometrial Carcinoma

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Recently, an activating mutation of the SRC gene has been implicated in about one-tenth of advanced colon cancers. The SRC 531 mutation results in truncation of SRC directly C-terminal to the regulatory Tyr 530 and appears to activate the Tyr 530. To investigate whether mutation of SRC plays an important role in the development and progression of gynecological tumors, we performed mutational analysis of the entire coding region of SRC in 70 ovarian carcinomas, 68 endometrial carcinomas and 3 endometrial stromal sarcomas by means of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by nucleotide sequencing and restriction fragment length polymorphism (RFLP) analysis. We found one truncated mutation at codon 531 (Gln to Stop) in an endometrial carcinoma. However, we found no mutation of this gene in ovarian carcinoma or endometrial stromal sarcoma. Our results suggest that mutation of SRC may be implicated in a small proportion of endometrial carcinomas.

Key words: SRC — Activating mutation — Endometrial carcinoma — Ovarian carcinomas

Studies of the mechanism of Src regulation have suggested that Src kinase activity is downregulated by phosphorylation of a critical carboxy-terminal tyrosine (Tyr 530 in human, Tyr 527 in chicken).1, 2) Mutation of the Tyr to Phe or deletion of the C-terminal regulatory domain resulted in promoting activity of c-Src protein.1–3) Irby et al. reported that a truncated mutation at codon 531 in SRC exon 12 was found in 12% of cases of advanced human colon cancers with distant metastasis, and that the mutation was activating and promoted the malignant potential.4) Elevation of Src kinase activity has been detected in several types of cancer,5–9) such as colon cancer, breast cancer, lung cancer and ovarian carcinoma. Clinical study of gynecological cancers has demonstrated that despite recent improvements in early diagnosis, surgical techniques, and chemotherapy, a half or more of the patients who undergo operative resections die of recurrent disease or metastases resistant to conventional therapies.10) However, the molecular events underlying metastasis and recurrence remain to be clarified. Recently, Maruyama et al., reported elevation of Src kinase activity in endometrial stromal cells with decidualization, suggesting that c-Src was specifically involved in the signaling cascades mediated by ovarian hormone stimulation. Some endometrial carcinomas are associated with ovarian hormone stimulation.11) These observations suggest that genetic alterations at the negative regulatory region of SRC may be implicated in the development and/or progression of gynecological malignancies, such as ovarian and endometrial tumors. To investigate whether SRC mutation is involved in these tumors, we performed genetic analysis of this gene in 70 ovarian carcinomas, 68 endometrial carcinomas and 3 endometrial stromal sarcomas, using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by direct sequencing, and restriction fragment length polymorphism (RFLP) methods.

MATERIALS AND METHODS

DNA preparation Materials used in this study were obtained during surgical treatments at Sapporo Medical University. We examined 70 fresh frozen samples with ovarian carcinoma (stage I 34 cases, stage II 8 cases, stage III 23 cases, stage IV 5 cases), 45 fresh frozen and 23 paraffin-embedded samples with endometrial carcinoma (stage I 41 cases, stage II 9 cases, stage III 15 cases, stage IV 3 cases), 3 paraffin-embedded samples with endometrial stromal sarcoma. DNAs were extracted from fresh frozen samples according to methods described elsewhere,12) and from paraffin-embedded samples by using the DEXPAT kit (TaKaRa, Tokyo) system according to the manufacturer’s instructions. Histological diagnosis of each tumor was done according to the WHO classification,13) and clinical stage was determined according to the International Federation of Gynecology and Obstetrics.14, 15)

PCR-SSCP, direct nucleotide sequencing and RFLP analysis To screen variant sequences of the entire coding region of exons 2–12 in the SRC gene, SSCP analysis was performed with various primers (Table I).16, 17) In this study, each 20 µl reaction mixture contained 10× PCR

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buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl$_2$, TaKaRa), 25 pmol of primers, 50 ng of genomic DNA or 2 µl of crude extract, 0.2 mM of each dNTP, and 0.25 unit of TaKaRa Taq (a recombinant Taq DNA polymerase, TaKaRa). Reaction mixtures were heated to 94°C for 2 min, and then cycled 35 times; each cycle consisted of denaturation at 94°C for 30 s, annealing at 50–55°C (Table I) for 30 s, and strand elongation at 72°C for 30 s.

After PCR, 10 µl of reaction mixture was transferred into 40 µl of a loading buffer (90% formamide, 20 mM EDTA, bromophenol blue and 0.05% xylene cyanol). The samples were heated at 80°C for 5 min and then quickly cooled on ice. Ten microliters of each mixture was loaded onto 12% polyacrylamide gel (ratio of acrylamide/bis-acrylamide, 39:1). Electrophoresis was performed at 17°C. After electrophoresis, gels were stained with SYBR green II (FMC BioProducts, ME). Nucleotide sequences of aberrant PCR products were determined using an Applied Biosystems model 377 DNA sequencer (Perkin-Elmer Corp., CA) with a primer used for PCR and a Dye terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, CA). To detect SRC 531 mutation (CAG to TAG), we performed ScaI based RFLP assay as described previously.4)

RESULTS

To screen alterations of the SRC gene in 141 gynecological tumors, we performed PCR-SSCP analysis of the entire coding region of this gene. In one case (T-48), an aberrant SSCP pattern in exon 12 was detected. Nucleotide sequence analysis of this case revealed a nonsense mutation at codon 531 (CAG to TAG) that would result in truncation of SRC directly C-terminal to the regulatory Tyr 530 (Fig. 1). The somatic nature of the mutation was established by sequencing DNA derived from normal tissue of the same patient (data not shown), indicating that this mutation appeared to affect one of the two alleles. Furthermore, the mutation in T-48 detected by direct sequencing was confirmed by ScaI-based RFLP analysis. The PCR product corresponding to exon 12 from tumor T-48 and the normal counterpart of the same patient were digested with ScaI, as the mutation at codon 531 generated a ScaI restriction site. ScaI digestion of the tumor PCR product produced digested fragments (184 bp and 51 bp) as well as the intact product (235 bp) from the unaltered allele. In the normal counterpart, only the intact product from the normal allele was seen after digestion (Fig. 2). Furthermore we performed nucleotide sequencing of the entire coding region of exons 2–12 in all tumors, as PCR-SSCP analysis is not able to detect all genetic changes.18) Direct nucleotide sequencing revealed that these tumors, except a sample of endometrial carcinoma T-48, had no mutation in exon 12 of the SRC gene. The clinicopathological data were examined with regard to onset age, Federation of International Gynecologic Oncology (FIGO) stage, histology, and familial malignancies; the patient (T-48) was 43 years old, pre-menopausal, with stage IIIc endometrioid carcinoma, and there were no familial malignancies within the second-degree relatives.

Table I. Primers of Exons 2–12 of the SRC Gene

| Exon number | Forward | Reverse | Annealing (°C) |
|-------------|---------|---------|---------------|
| 2           | 5-CTG CCA GGA CCA TGG GTA GCA-3 | 5-GCG CAC TGA CCG GCC AGC G-3 | 55 |
| 3           | 5-CTC TCT GCA GGT GTA GTG AC-3 | 5-CCA ACT CCT CAG CCT GGA TA-3 | 54 |
| 4           | 5-CTT CTT CAG AGA GGA GAG GTA CAA-3 | 5-CCA ACT CAG AGA GGA G-3 | 50 |
| 5           | 5-CTT CTT CAG AAC ACG CCG A-3 | 5-GTA CCT TCC GTC TCA GTG CT-3 | 53 |
| 6           | 5-GGA CCG CAG GTG ACT ATT TGG G-3 | 5-GCT TCC CCA CCT GGA TG-3 | 54 |
| 7           | 5-CTT CTT CAG AAC ACG CCG A-3 | 5-GTA CCT TCC GTC TCA GTG CT-3 | 52 |
| 8           | 5-GTA CCT CCA CCT GGA TA-3 | 5-CTC ACC CCT GTA GTA CT-3 | 50 |
| 9           | 5-GCT GCC CAG GGA GTT TGC TG-3 | 5-ACC ACC GGG AAT TGC TC-3 | 54 |
| 10          | 5-CTG CAG ATC GGC TCA GGA AT-3 | 5-ACC ACC TTG CCG CGC GGT G-3 | 53 |
| 11          | 5-CTC CCG ATC CAG GGA TG-3 | 5-ACC ACC TTG CCG CGC GGT G-3 | 54 |
| 12          | 5-GGA CCA CAG GGA TGG GTA AC-3 | 5-GGA CCA CAG GGA TGG GTA AC-3 | 55 |

Fig. 1. SRC mutation in endometrial tumors. Arrows indicate nucleotide change. CAG to TAG at codon 531 in case T-48.
DISCUSSION

In this study, we performed genetic screening of SRC in 141 primary gynecological tumors, as an activating mutation has been identified in about one-tenth of advanced colon cancers. We found only one truncated mutation (T-48), which was the same genetic alteration as that identified in colon cancer. This case (T-48) was a pre-menopausal patient with endometrial carcinoma, which appeared to be associated with ovarian hormone stimulation. This patient (T-48) with SRC 531 mutation had an extensive tumor over the uterus in the pelvic cavity with pelvic lymph-node metastasis. However of 68 endometrial carcinomas examined, only 18 were advanced types (stage III–IV) and 23 patients had pre-menopausal status. Previous studies demonstrated that SRC is activated not only by genetic alteration, but also through other pathways such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). Thus, to clarify the possible role of SRC somatic mutation, large numbers of endometrial carcinomas including advanced types and/or pre-menopausal status should be screened.

These observations do not completely exclude the possibility of SRC being activated through genetic alterations, as the mutation screening was very limited and the approach used does not allow the detection of some mutation types, such as methylation and/or large genomic deletion. Mutational analysis should be performed to evaluate other types of genetic alteration. Our results suggest that mutation of the SRC gene may make a limited contribution to the development and/or progression of a small proportion of endometrial carcinomas.

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