

Endometrial carcinoma in a breast cancer patient treated with tamoxifen: activation of K-ras proto-oncogene

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Abstract. The use of tamoxifen for breast cancer therapy is linked to an increased danger of developing endometrial neoplasias in postmenopausal patients receiving the drug. Understanding the molecular mechanisms of the tumorigenic activity of tamoxifen may be of great prognostic and therapeutic significance. Our study suggests that tamoxifen treatment and alterations of the K-ras proto-oncogene may occur as parallel events in carcinogenesis of the endometrium.

Introduction

Tamoxifen, a non-steroidal compound, is widely used in therapy of estrogen-receptor rich breast carcinoma, because it acts as an agonist and down-regulates the stimulatory effect of the hormone-receptor complex. In addition, it alters the cellular metabolism of estradiol to less growth stimulating compounds (1). Use of the drug is proved to significantly reduce recurrence from early stages of the disease and the risk of a new primary cancer development (2). However, since postmenopausal patients are relatively hypoestrogenic, tamoxifen may stimulate endometrial growth.

A number of cases of endometrial carcinoma associated with the use of tamoxifen have been reported (3,4). This effect is thought to be due to estrogenic stimulation of the uterine endometrium by the anti-estrogen, however, the molecular mechanisms of the neoplasia caused by tamoxifen are still unclear. Experimental results provide evidence for transcriptional activation of *c-fos* and *c-jun*-D proto-oncogenes by tamoxifen (5), which may result in alterations in AP-1 levels and in abnormal expression of a variety of AP-1 regulated genes. *ras* gene contains AP-1 responsive elements (6). Moreover, *c-jun* and *fra1* are crucial mediators of the *ras*-transformation process (7).

The Ras proteins play a central role in regulating cell growth and their mutations can lead to abnormal proliferation and carcinogenesis (8), usually in combination with additional oncogenic events, e.g viral infections (9).

We report a case of endometrial adenocarcinoma in a breast cancer patient treated with tamoxifen. Using PCR-RFLP analysis, we detected a mutation of the K-ras proto-oncogene in the endometrial tumor tissue, thus suggesting that tamoxifen may exert its neoplastic effect in the uterus through mutational activation of cellular oncogenes.

Case report

An 81-year-old woman underwent a right radical mastectomy for an infiltrating carcinoma in 1978. Bone scintigraphy detected positive areas of metastases, as well. She was treated with tamoxifen as adjuvant therapy for 13 years (10 mg, three times daily). She had a diagnostic curettage for metrorrhagia in April 1991 and the histological report indicated an endometrial adenoacanthoma, Grade II, moderately differentiated. The patient underwent total abdominal hysterectomy with bilateral oophorectomy (FIGO Stage 1B) and died a few weeks later.

Materials and methods

DNA extraction. Three 10 µm thick sections from each formalin-fixed, paraffin-embedded tissue sample were deparaffinized by xylene, followed by an ethanol wash, lysed in a solution containing 10 mM Tris-HCl pH 8.0, 25 mM EDTA, 100 mM NaCl, 0.5% SDS and 100 µg/ml proteinase K, and incubated at 60°C, for 24 h. The samples were then extracted with phenol, phenol/chloroform and chloroform and DNA was ethanol precipitated and resuspended in 30 µl of double distilled water. Samples were: endometrial tumor tissue from the breast cancer patient previously treated with tamoxifen and adjacent normal tissue from the same patient.

Primers and PCR amplification. The oligonucleotide primers used for detection of K-ras codon 12 mutations have been previously described (10).

One µl of the extracted DNA sample, or two µl of the control SW480 cell line encompassing K-ras codon 12 mutation (10), were amplified in a reaction mixture of 50 µl,

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containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 3.5 mM MgCl₂, 200 μM of each dNTP, 1 μM of each primer and 2.5 U Taq polymerase (Perkin Elmer, Roche Molecular Systems, Inc., USA). The mixture was heated for 5 min at 98°C before addition of the polymerase, and then subjected to 35 cycles of amplification: denaturation at 94°C for 50 sec, annealing at 58°C for 45 sec and elongation at 72°C for 50 sec, increasing the elongation time 1 sec per cycle.

RFLP analysis. 20 μl aliquots of the amplification products were digested with 50 U of BstNI (New England Biolabs, UK), for 3 h, at 60°C. *K-ras* codon 12 positive samples give a 142 bp band after digestion, whereas negative samples produce a 113 bp band (10).

Results and Discussion

K-ras codon 12 point mutations are implicated in the development of primary endometrial carcinomas (10). Using PCR-RFLP analysis, we detected a *K-ras* codon 12 mutation in the endometrial tumor tissue of the breast cancer patient previously treated with tamoxifen, whereas no point mutation was detected in the adjacent normal tissue (Fig. 1). Lane 1 represents undigested PCR product (157 bp) and lane 2 a positive control SW480 cell line, homozygous for *K-ras* codon 12 mutation (10). Lane 3 represents the adjacent normal endometrial tissue of the patient and the 113 bp band is clearly shown. The endometrial tumor tissue PCR-RFLP product is electrophoresed in lane 4. A light 142 bp band is shown, demonstrating that the sample is homozygous for *K-ras* codon 12 mutation. The 113 bp band in lane 4 is due to the normal tissue present in the sections (if the sample was heterozygous, the 142 bp and the 113 bp bands should have the same intensity).

In a previous study, we reported that *K-ras* mutations are implicated in endometrial cancer of the Greek population in 15% of cases (8/55) (10). Among the 55 patients tested, the case reported here was the only one previously treated with tamoxifen. Although only one patient with endometrial cancer who had received tamoxifen was examined, the low background rate (12.7%, 7/55) of *K-ras* point mutations detected in endometrial carcinomas of the Greek population, enables us to suggest a correlation between the use of tamoxifen and endometrial carcinogenesis.

Tamoxifen is a drug with successful application in the treatment of primary breast cancer. However, it has estrogen agonist activity in the endometrium of postmenopausal women and creates an environment which stimulates cell division and growth of the target organ (11). Induction of proto-oncogene expression was reported as a mechanism of endometrial tumorigenesis associated with chronic tamoxifen treatment (5). We report mutational activation of *K-ras* proto-oncogene as an additional mechanism of tamoxifen-induced carcinogenesis. Since tamoxifen induces elevated rates of cellular growth and division, we propose that endometrial cells have a predisposition to develop mutational alterations in cellular oncogenes, for example due to DNA polymerase errors or in addition to a second tumorigenic event, e.g. a mutagen, thus leading to an increased oncogenic potential.

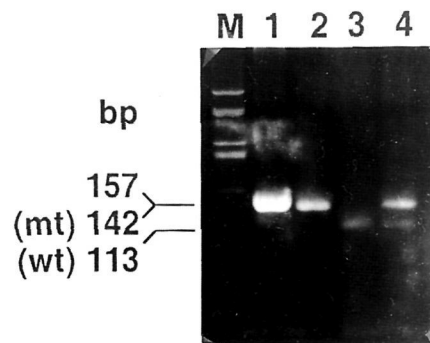


Figure 1. Detection of *K-ras* codon 12 point mutations by PCR-RFLP analysis. PCR products were digested with BstNI restriction endonuclease and electrophoresed through a 4% agarose gel. Lane 1, undigested PCR product, 157 bp; lane 2, positive control (SW480 cell line), 142 bp; lane 3, negative sample (normal tissue), 113 bp; lane 4, positive sample (tumor tissue), 142 bp. Lane M, molecular weight marker pUC18/ HaeIII. mt, mutation; wt, wild-type.

Association between endometrial cancer and therapy with tamoxifen cannot be ignored (3). Vaginal and endometrial cytology, vaginal sonography and a strict gynecological follow-up were proposed to prevent this side effect of the drug (3). Combination of these methods with molecular techniques, for example detection of proto-oncogene mutations, may contribute to the earlier diagnosis of the disease and provide alternative methods of gene therapy.

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