Genomic Changes in Endometrial Polyps Associated with Tamoxifen Show No Evidence for Its Action as an External Carcinogen

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Abstract

Eighty-eight endometrial specimens from 36 postmenopausal breast cancer patients treated with tamoxifen were investigated cytogenetically and molecularly using fluorescence in situ hybridization with appropriate probes for the HMGIC and HMGIY genes. Twenty control specimens, 10 endometrial polyps, and 10 endometrial biopsy specimens were investigated in the same way. Of the 88 specimens, 44 were from endometrial polyps; 7 were from endocervical polyps; 20 were from cystic endometrium; 30 were from normal or atrophic endometrium, normal endocervix, or myometrium; and 4 were from endometrial carcinomas. Cytogenetic investigation of the endometrial polyps showed the nature of the chromosome changes in tamoxifen-induced polyps to be the same as that in the controls and in sporadic endometrial polyps described in the literature. HMGIC and HMGIY gene rearrangements in both groups were identical as shown by fluorescence in situ hybridization, which also allowed for the detection of seven hidden paracentric inversions involving 12q15, one of which occurred in a cystic endometrium. The carcinomas did not exhibit any of these changes. Because abnormal expression of HMGIC or HMGIY as a consequence of structural chromosome changes in 12q15 or 6p21, respectively, is invariably associated with benign neoplasia, tamoxifen-associated endometrial polyps are unlikely to undergo further malignant transformation, and a mode of action of tamoxifen as an external carcinogen is unlikely.

Introduction

Tamoxifen is a nonsteroidal triphenylethylene derivative that has been widely used in the treatment of breast cancer since the 1970s. It blocks the growth-promoting effects of estrogens in breast tissue, mainly through competitive inhibition of the estrogen receptor mechanism (1).

In addition, an estrogenic effect on the endometrium has been described, resulting in a variety of endometrial changes ranging from endometrial hyperplasia to polyps and endometrial cancer (2–4). Most sporadic endometrial polyps present discernable genomic changes. Cytogenetic investigations demonstrated that different specific chromosome rearrangements can be observed despite a seemingly identical clinical and morphological appearance. Besides a numerically important group with a normal karyotype, three major cytogenetically abnormal subgroups have been identified involving the 6p21, 12q15, and 7q22 regions (5). These chromosome abnormalities led to the discovery of underlying molecular rearrangements and to partial elucidation of the mechanisms of tumorigenesis by abnormal expression of the HMGIC and HMGIY genes in endometrial polyps with 12q and 6p anomalies, respectively (6–8).

Because the frequency of endometrial polyps significantly increases among tamoxifen-treated patients (9, 10), we investigated these endometrial changes cytogenetically and molecularly to examine whether the same genomic changes were present in the tamoxifen-related tumors as those known to occur in endometrial changes unrelated to tamoxifen.

Materials and Methods

Patients and Clinical Data. Transvaginal ultrasonography combined with sonohysterography, when necessary, was the technique used in our monitoring of the uterus in 36 tamoxifen-treated postmenopausal breast cancer patients (some clinical data are summarized in Table 1; Ref. 11).

Suspected endometrial lesions were resected together with a randomly taken endometrial biopsy. Pathological evaluation of each specimen was performed by the same pathologist. A total of 88 specimens from tamoxifen-treated patients were studied cytogenetically (Table 2) in addition to 20 specimens from the control population.

Cytogenetic and Molecular Cytogenetic Investigations. G-banded metaphases were obtained after short-term culture of an overnight disaggregated specimen of each sample according to routine methods. At least 20 metaphases were analyzed in each tissue sample.

FISH1 was performed after G-banding of the same metaphase spread according to the procedure described by Kleivi et al. (12). Metaphases were hybridized with a pool of cosmids 27E12 and 142HI flanking the third intron of the HMGIC gene mapped at 12q15 (6) and a PAC clone containing the HMGIY gene located at 6p21.2 (13), respectively. Slides were analyzed using a Zeiss Axiosplan fluorescence microscope (Zeiss, Oberkochen, Germany).

Results were processed and recorded with the Power Gene Karyotyping System (Perceptive Scientific Instruments; Haleddahle, United Kingdom). In translocations affecting HMGIC or HMGIY, split signals were observed on the derivative chromosome 12 or 6, respectively, and on the translocation chromosome partners. In the case of pericentric or paraacentric inversion of chromosome 12 or 6, split signals on the short and the long arm or a double signal on one arm, respectively, were observed on those chromosomes. In cases without an intragenic break in the HMGIC or HMGIY genes, the signal was not found to be split in its original site or on the translocation partner.

Results

Pathology Findings. Of the resected endometrial lesions from tamoxifen-treated patients (88 specimens), 44 specimens were diagnosed as endometrial polyps, whereas 3 specimens were endocervical polyps. Results of the histological investigation of the 41 other specimens are found in Table 2.

Ten samples of normal-looking endometrium from control patients

1 The abbreviations used are: FISH, fluorescence in situ hybridization; PAC, PI artificial chromosome.
were histologically fully normal. Their endometrial polyps (n = 10) had a typical morphology.

**Cytogenetic Investigations.** Of 88 specimens from the 36 tamoxifen-treated patients, 80 specimens were successfully karyotyped. Karyotype failures were observed in one polyp, one cystic endometrium, one endometrial carcinoma, and five histologically normal endometria. Twenty-two of 80 specimens had clonal chromosomal aberrations: 21 of these specimens were endometrial polyps; and 1 specimen was a seropapillary carcinoma. Among these 22 karyotypically abnormal samples, 8 samples had aberrations involving chromosome 6 with breaks in the region 6p21, whereas another 8 samples showed anomalies of chromosome 12 clustering in the 12q14–15 region. All of these samples were polyps. Five more polyps had other clonal chromosomal changes, and the only chromosomally abnormal carcinoma had a very complex karyotype (near 3N).

In the controls (n = 10), three polyps had involvement of 6p21, three polyps had involvement of 12q15, and four polyps had a normal karyotype. All endometria (n = 10) were cytogenetically normal.

**Molecular Cytogenetic Investigation.** In the eight polyps with involvement of 6p21, the signal was split in seven polyps. In six of these polyps, a signal was found on each derivative chromosome (Fig. 1, a and b). In one case, one signal was found on 6p, and one signal was found on 6q, belonging to the same chromosome 6, due to a pericentric inversion.

In one case (case 26, polyp 3), the signal was not split but instead had moved to translocation partner chromosome 15, indicating a break outside the HMGIY gene (Fig. 1, e and f).

In the eight polyps with involvement of 12q15, the signal was split in seven polyps. In six of these polyps, a signal was found on each derivative chromosome. In one case, there was a split signal on one chromosome 12 due to a pericentric inversion in that chromosome.

In the remaining polyp, no splitting was found in a pericentric inversion of chromosome 12, indicating a break outside the HMGI gene. No signal splitting was observed in the five cases with other clonal chromosome abnormalities. One remaining case showed two to four double minute chromosomes in which a chromosome 12 segment including 12q15 was amplified (14).

In seven additional cases (six endometrial polyps and one cystic endometrium) with an apparently normal karyotype, FISH with the cosmid pool of HMGIC revealed hidden paracentric inversions in 12q (Fig. 1, c and d). None of the cases with HMGIC rearrangements showed HMGIY aberrations and vice versa. All control cases exhibited the expected signal distribution (i.e., a split signal on each derivative chromosome in the cases with 6p and 12q involvement). No hidden inversions could be detected in this group. The three carcinomas, one with an abnormal karyotype and two with normal karyotypes, showed no splitting of the signals.

**Discussion**

It is demonstrated in this study that in endometrial polyps occurring in breast cancer patients treated with tamoxifen, the same types of chromosomal and gene rearrangements are found as in endometrial polyps unrelated to tamoxifen treatment. This observation is important for several reasons:

(a) Some evidence has been accumulated that tamoxifen and tamoxifen-like compounds may be carcinogenic and as such may be responsible for the occurrence of neoplastic growth in tamoxifen-treated patients, ranging from hyperplastic endometrium to endometrial polyps and cancer. The evidence is based on clinical observations, DNA toxicity studies, and experiments on animals. As a consequence, tamoxifen was recently classified in a comprehensive IARC study as a class I carcinogen (15). If tamoxifen was a classical carcinogen with toxicity for DNA, as the above-quoted studies suggest, and if endometrial hyperplasia and polyps were in some cases only initial stages of a stepwise process leading to overt malignancy, then one would expect a genomic study of these polyps to show chromosomal and gene rearrangements different from those found in patients not treated with tamoxifen. Tamoxifen-associated endometrial carcinomas should then show the chromosomal and/or gene rearrangements of the initial not fully malignant stages on which other changes related to tumor progression may or may not have been superimposed. However, data obtained in this study clearly show that the nature of chromosome changes and gene rearrangements in tamoxifen-associated polyps and in controls is the same, and that none of these are found in the tamoxifen-associated carcinomas. In the latter, as in non-tamoxifen-associated carcinomas (16), a normal or a very complex karyotype with few normal chromosomes were found, and no HMGI gene rearrangements were detected by FISH. At the other end of the spectrum, our case of cystic endometrium with hidden paracentric inversion affecting 12q15 and a corresponding HMGIC intragenic break could represent an early stage of a developing endometrial polyp.

(b) The HMGI and HMGIY genes code for so-called architectural proteins that influence chromatin structure and gene transcription [reviewed in Wunderlich and Böttger (17)]. Breaks in 12q15 or 6p21, discernable morphologically (translocations, pericentric inversions) or only detectable by the appropriate FISH technique (paracentric inversions) in the chromosomes of endometrial polyps, seem to occur at the molecular level mostly within the large third intron of the HMGI gene or, more rarely, at some distance outside the gene (6) and inside or 3' outside the gene for HMGIY (8). All of these possible types of genomic rearrangements were encountered in the present study. All are known to lead to abnormal expression of the gene in question. Furthermore, not only endometrial polyps but also a whole series of mesenchymal tumors (uterine leiomyoma, mixed salivary gland tumor, pulmonary chondroid hamartoma, lipoma, fibroadenoma, and hamartoma of the breast) share this HMGI and/or HMGIY involvement (18). However, all of them are invariably benign, and they have not been found to be the initial stages of true malignancies. Endometrial polyps, whether sporadic or tamoxifen associated, presenting HMGC or HMGI rearrangements leading to abnormal expression of these genes thus apparently follow identical genetic pathways that clearly do not seem to lead to malignancy. Whether this also holds true for the minority of cases with other chromosome changes in sporadic as well as in tamoxifen-associated polyps and for the impor-

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<tr>
<th>Table 1</th>
<th>Some clinical data on the 36 tamoxifen-treated patients</th>
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<tr>
<td>Age (yr)</td>
<td>62.3 47-85</td>
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<tr>
<td>Parity</td>
<td>2.4 0-7</td>
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<tr>
<td>Years postmenopausal</td>
<td>12.5 0-34</td>
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<tr>
<td>Years after breast cancer</td>
<td>4.7 0-17</td>
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<tr>
<td>Months of tamoxifen use</td>
<td>33.9 3-113</td>
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<tr>
<td>Daily dose (mg)</td>
<td>30 20-40</td>
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<tr>
<td>Total cumulative dose (mg)</td>
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<th>Table 2</th>
<th>Pathology findings in 88 resected endometrial specimens for 36 tamoxifen-treated patients</th>
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<tr>
<td>Endometrial polyps</td>
<td>44</td>
</tr>
<tr>
<td>Endocervical polyps</td>
<td>3</td>
</tr>
<tr>
<td>Cystic endometrium</td>
<td>7</td>
</tr>
<tr>
<td>Atrophic endometrium</td>
<td>3</td>
</tr>
<tr>
<td>Normal endometrium</td>
<td>23</td>
</tr>
<tr>
<td>Normal myometrium</td>
<td>2</td>
</tr>
<tr>
<td>Normal endocervix</td>
<td>2</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Seropapillary adenocarcinoma</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>88</td>
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NO ROLE SHOWN FOR TAMOXIFEN AS AN EXTERNAL CARCINOGEN

Fig. 1. a and b, metaphase spread after FISH of an endometrial polyp cytogenetically described as t(6;20)(p21;q13). FISH performed with a pool of PACs 8603 and 8605 (both containing HMGIC) showed hybridization signals on normal-looking chromosome 6 and on both derivative chromosomes der(6) and der(20) (a, arrows), thus indicating a rearrangement of HMGIC or its flanking sequences. The same metaphase spread after GTG-banding shows normal-looking chromosome 6 and the derivative chromosomes der(6) and der(20) (b, arrows). c and d, metaphase spread after FISH on an endometrial polyp with a normal karyotype. FISH with cosmids 27E12 and 142H1 flanking the third intron of HMGIC indicated one hybridization signal on normal-looking chromosome 12 and two split hybridization signals on der(12) due to a paracentric inversion of 12q (c, arrows), and the same metaphase spread is shown after GTG-banding. Both normal-looking chromosomes 12 are indicated by arrows (d). e and f, metaphase spread after FISH of an endometrial polyp with karyotype 46,XX,t(6;20)(p21;q13)/46,XX using PACs 8603 and 8605 containing HMGIC. Hybridization signals on normal chromosome 6 and der(15) reveal that HMGIC is translocated to der(15) (e, arrows). The same metaphase spread is shown after GTG-banding. Normal-looking chromosome 6 and der(15) are indicated by arrows (f).

tant subgroup without chromosome changes and without HMGIC or HMGIC rearrangement is unknown. Tamoxifen-induced pathological changes of the endometrium are believed by some to present "an overlapping pathological spectrum ranging from generalized simple endometrial hyperplasia and hyperplastic polyps to polyp-cancers and primary invasive malignancies of the endometrium" (4). This statement may not be valid for tamoxifen-induced endometrial polyps with 12q15 or 6p21 involvement. In this group, a mode of action for
tamoxifen as an external carcinogen is very unlikely on the basis of the present study.

This study was planned to evaluate genomic changes in endometrial polyps occurring in women treated with tamoxifen and in appropriate controls. As seen in Table 1, the mean duration of tamoxifen treatment was 33.9 months (range, 3–113 months); thus, the possible occurrence of endometrial carcinomas after a longer period of treatment cannot be excluded.

Acknowledgments

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References

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