[CANCER RESEARCH 58, 2278–2281, June 1, 1998]

Advances in Brief

Genomic Changes in Endometrial Polyps Associated with Tamoxifen Show No Evidence for Its Action as an External Carcinogen[1]

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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; 3 were from endocervical polyps; 7 were from cystic endometrium; 30 were from normal or atrophic endometrium, normal endocervix, or myometrium; and 4 were from endometrial carcinomas. Chromosome 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. Part of each specimen was processed for pathological investigations, and the remaining tissue was used for cytogenetic analysis.

For the purpose of this study, endometrial polyps as well as normal-looking endometrium from 10 patients who were not treated with tamoxifen were resected and used as controls. 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.

FISH[3] was performed after GTG-banding of the same metaphase spreads according to the procedure described by Kievits *et al.* (12). Metaphases were hybridized with a pool of cosmids 27E12 and 142H1 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 Axioplan fluorescence microscope (Zeiss, Oberkochen, Germany). Results were processed and recorded with the Power Gene Karyotyping System (Perceptive Scientific Instruments; Halleddale, 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 paracentric 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

Received 2/17/98; accepted 4/17/98.

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[1] This article presents research results of the Belgian program on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors.

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[3] The abbreviations used are: FISH, fluorescence *in situ* hybridization; PAC, P1 artificial chromosome.

Table 1 *Some clinical data on the 36 tamoxifen-treated patients*

	Mean	Range
Age (yr)	62.3	47–85
Parity	2.4	0–7
Years postmenopausal	12.5	0–34
Years after breast cancer	4.7	0–17
Months of tamoxifen use	33.9	3–113
Daily dose (mg)	30	20–40
Total cumulative dose (mg)	31918	1800–115200

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 endometriums. 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 endometriums (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 *HMGIC* 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 *HMGIC* 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 *HMGIC* 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 *HMGIC* 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 *HMGIC* or *HMGIY* 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-

Table 2 *Pathology findings in 88 resected endometrial specimens for 36 tamoxifen-treated patients*

Endometrial polyps	44
Endocervical polyps	3
Cystic endometrium	7
Atrophic endometrium	3
Normal endometrium	23
Normal myometrium	2
Normal endocervix	2
Endometrial carcinoma	2
Poorly differentiated carcinoma	1
Seropapillary adenocarcinoma	1
Total	88

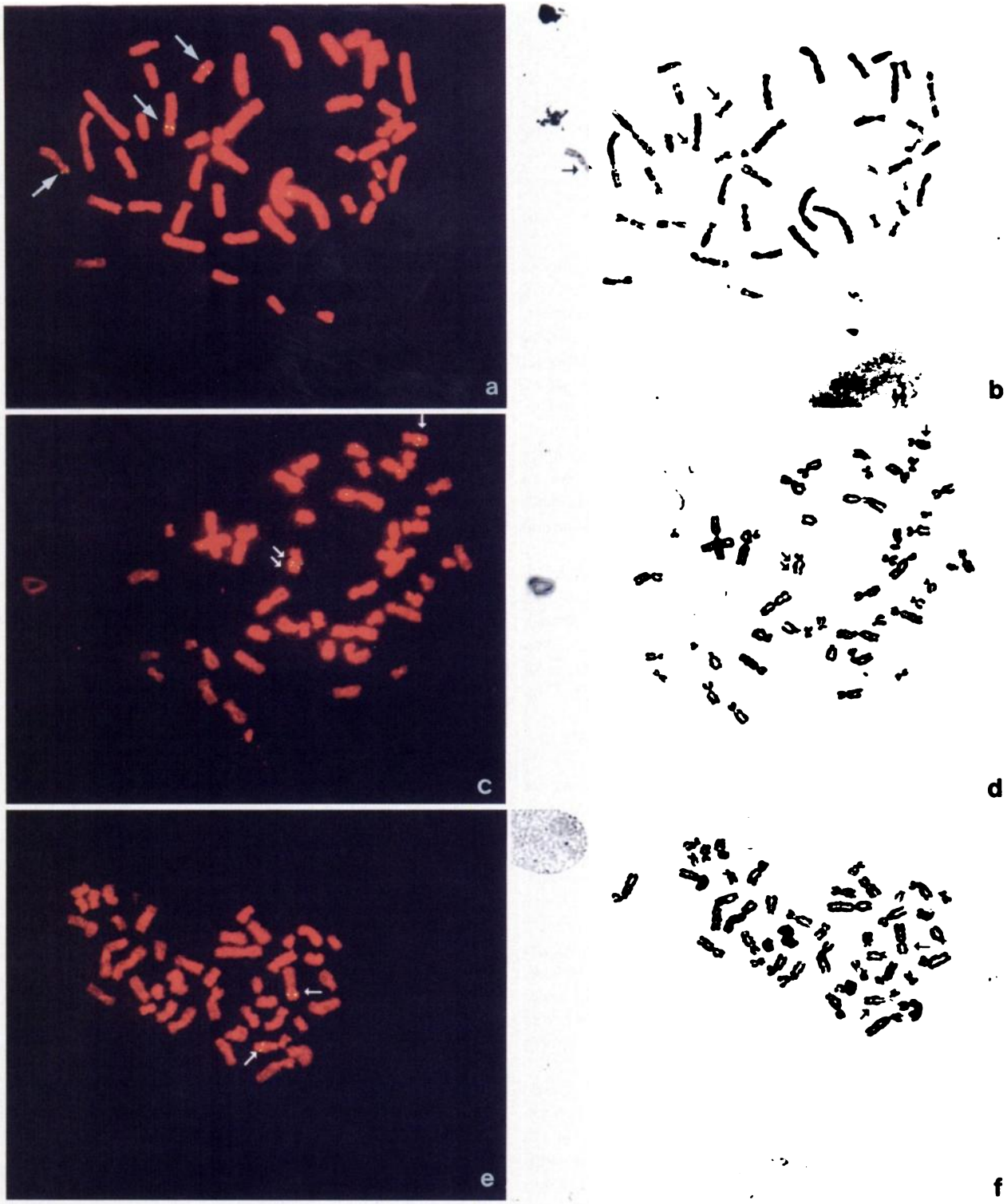

Fig. 1. *a* and *b*, metaphase spread after FISH of an endometrial polyp cytogenetically described as t(6;20)(p21;q13). FISH performed with a pool of PACs 8603 and 8605 (both containing *HMGIY*) showed hybridization signals on normal-looking chromosome 6 and on both derivative chromosomes der(6) and der(20) (*a*, *arrows*), thus indicating a rearrangement of *HMGIY* 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;15)(p21;q21)/46,XX using PACs 8603 and 8605 containing *HMGIY*. Hybridization signals on normal chromosome 6 and der(15) reveal that *HMGIY* 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 *HMGIY* 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

We thank Lut Mekers and Belinda Carleer for technical assistance and Rita Logist for clerical assistance.

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