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

Paola Dal Cin, Dirk Timmerman, Ivo Van den Berghe, Sylke Wanschura, Bernd Kazmierczak, Ignace Vergote, Jan Deprest, Patrick Neven, Philippe Moerman, Jörn Bullerdiek, and Herman Van den Berghé

Center for Human Genetics [P. D. C., H. V. d. B.] and Departments of Obstetrics and Gynecology [J. T., J. V., J. D.] and Pathology [J. V. d. B., P. M.], University of Leuven, B-3000 Leuven, Belgium; Department of Obstetrics and Gynecology, St. Jansziekenhuis, B-1000 Brussels, Belgium [P. N.]; and Center for Human Genetics and Genetic Counseling, University of Bremen, D-28359 Bremen, Germany [S. W., K. B., J. B.]

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 HMG1Y 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 HMG1Y 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 HMG1Y as a consequence of structural chromosome changes in tamoxifen-induced polyps, it is unlikely that tamoxifen-induced polyps are caused by a direct DNA damage event.
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 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 cosmids 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:
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 HMGIY 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).

A specific subgroup without chromosome changes and without HMGI 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.

References

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