



Cell death evaluation in benzo[*a*]pyrene-transformed human breast epithelial cells after microcell-mediated transfer of chromosomes 11 and 17

Maria Luiza S. Mello^{a,*}, Luis Fernando Barbisan^b, Mohamed H. Lareef^c,
Jose Russo^c, Benedicto de Campos Vidal^a

^a Department of Cell Biology, Institute of Biology, UNICAMP, 13084-971 Campinas (SP), Brazil

^b Department of Morphology, Institute of Biosciences, UNESP, 18618-000 Botucatu (SP), Brazil

^c Fox Chase Cancer Center, Philadelphia, PA 19111, USA

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Abstract

The incidence of apoptosis and nuclear instability, including the incidence of catastrophic death, were investigated in benzo[*a*]pyrene (BP)-transformed human breast epithelial cells (BP1-E cell line) after microcell-mediated transfer of chromosomes 11 and 17. Since the introduction of normal chromosomes 11 and 17 into tumorigenic human breast BP1-E cells reverts some of these cells' characteristics (especially those affected by microsatellite instabilities and loss of heterozygosity) to those of parental non-transformed MCF-10F cells, it was expected that the cell death rates would also be affected by this treatment. The transfer of the mentioned chromosomes, especially chromosome 17, to tumorigenic BP1-E cells increased the apoptotic ratios and decreased the nuclear instability ratios, thus showing that the microsatellite instability and loss of heterozygosity induced by BP in these chromosomes of MCF-10F cells affect the control of cell death mechanisms.

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1. Introduction

Spontaneously immortal human breast epithelial cells (MCF-10F) subjected to treatment with benzo[*a*]pyrene (BP) have yielded cell lines with gradual steps of neoplastic progression [1–3]. Microsatellite instability in chromosomes 11 and 17, loss of heterozygosity in chromosome 17, as well as alterations in some other chromosomes have been associated

with the neoplastic progression in these cells [4–9]. Apoptosis and catastrophic death have been observed in MCF-10F cells and in the cell lines obtained from these after transformation with BP [3]. The apoptotic ratios tend to decrease whereas multinucleate and micronucleate images resulting from catastrophic death tend to increase with BP transformation in MCF-10F cell lines [3]. In contrast to the relatively rapid process involved in apoptosis, catastrophic death is a phenomenon which results from a slow process of cell enlargement and G2 arrest followed by aberrant mitosis and relocation of the nuclear membrane around chromosomal fragments or masses [10–12].

* Corresponding author. Tel.: +55-19-289-3124;

fax: +55-19-3788-6111.

E-mail address: mlsmello@unicamp.br (M.L.S. Mello).

When normal chromosomes 11 and 17 are introduced into BP-transformed MCF-10F cells (BP1-E cell line) by the microcell-mediated chromosome transfer (MMCT) assay, some of the characteristics of these cells revert to those of non-transformed MCF-10F cells. These characteristics include a decrease in cell growth, a reduction in colony efficiency, colony size and telomerase activity, and a tendency for the frequency distribution of DNA amounts and nuclear sizes to be restored to values typical for non-transformed MCF-10F cells [9,13]. Some of these characteristics revert or are drastically reduced only in the presence of normal chromosome 17 [9,13].

Since changes involving cell growth are restored partly or totally in the presence of normal chromosomes 11 and 17, it would be expected that cell death rates could also be affected under such experimental conditions, if correspondingly affected chromosomes were responsible for steps of this process. Indeed, in human breast epithelial cells *in vitro* normal chromosome 17 at region p13.1 has been reported to play a part in the activation of the FAS receptor, which mediates apoptosis [14]. In the present study, apoptosis and parameters that reflect nuclear instability, including catastrophic death, were thus investigated in BP1-E cells which received normal chromosomes 11 and 17 by MMCT.

2. Material and methods

2.1. Cells

Non-transformed MCF-10F cells at passage 130 and tumorigenic BP1-E cells obtained by treating MCF-10F cells with benzo[*a*]pyrene (0.2 µg/ml) were used. BP1-E cells received normal chromosomes 11 and 17 by MMCT. The cells were transfected with the plasmid pSV2neo using the Calphos maximizer transfection protocol (Clontech, Palo Alto, CA) and then fused with microcells generated from human chromosome donor cells (A9-11neo or A9-17neo) to produce the microcell hybrids BP1E-11neo and BP1E-17neo, respectively. Colonies surviving in DMEM medium containing G-418 (400 µg/ml) were subcloned. BP1-E cells at passage 45, and BP1E-11neo and BP1E-17neo cells at passage 7 were used. Since BP1-E and

BP1E-11neo grew faster than BP1E-17neo, BP1-E and BP1E-11neo cells were grown for 48 h and MCF-10F and BP1E-17neo cells for 96 h, in order to obtain the same level of confluence (80%). Three clones of cells containing chromosome 11 or 17 were used.

BP1-E cells have 46 chromosomes like parental MCF-10F cells [1]. In chromosome transfected cells an extra chromosome 11 or 17 could be identified. Since the donor chromosome was tagged with neomycin in its centromeric region, if the cells were not transfected with the new chromosome they could not survive in the growth medium that contained neomycin. After the chromosome transfer, a FISH assay was performed to identify the extra chromosome which had been transfected, in a similar way as that reported previously [8]. Additionally, one of us (MHL) conducted simple sequence repeat analysis (gel method) to identify the loci transferred, by using several primers and distinguishing the entire donor chromosome [21]. All these allow concluding that the donor chromosome had been transferred. Chromosomes other than those mentioned above were not observed after MMCT.

2.2. Cell preparations and staining

All the cells were grown on Permaxox[®] plastic well slides (Electron Microscopy Sciences, Fort Washington, USA) and fixed in an absolute ethanol–acetic acid mixture (3:1, v/v) for 5 min, then air dried at room temperature and subjected to the Feulgen reaction in which acid hydrolysis was done with 4 M HCl for 75 min at 25 °C. The preparations were subsequently treated with acid fast green solution recommended for cytochemistry of total proteins [15].

2.3. Analysis of cellular parameters

Cells prepared as described above were used to evaluate the apoptotic ratios, catastrophic cell death ratios, mononucleate giant cells, micronucleate cells, anomalous mitosis, and the nuclear instability ratio (the sum of cells undergoing catastrophic death and anomalous mitosis, plus mononucleate giant and micronucleate cells) in approximately 7500 cells for each transformed cell type (~2500 cells per slide).

Apoptotic and catastrophic cell death ratios were evaluated in 6000 non-transformed MCF-10F cells (2000 cells per slide). The morphological identification of apoptosis and catastrophic death was based on previously reported criteria, i.e., shrunken interphase nuclei with or without the formation of apoptotic bodies indicated apoptotic death whereas cells containing multiple micronuclei with no chromatin condensation were indicative of “mitotic or catastrophic death” [3,12,16,17]. Micronuclei were identified and analyzed as described by Tolbert et al. [18] and Fenech [19]. Counts were done in a Nikon photomicroscope equipped with 60× objective and a measuring ruler. The results were compared by Goodman’s test [20] for contrasts among multinomial proportions.

3. Results

Apoptosis and catastrophic death were verified in all of the cell lines studied here (Fig. 1). However, the apoptotic ratios increased significantly after the transfer of the normal chromosomes (especially chromosome 17) to tumorigenic BP1-E cells (Table 1). In the case of chromosome 17 being transferred, the apoptotic ratio practically reverted to the value for non-transformed MCF-10F cells [3]. In addition, the nuclear instability ratio, which represents the sum of cells undergoing catastrophic death and anomalous mitosis plus mononucleate giant cells and micronucleate cells, decreased significantly with the insertion of normal chromosomes (Table 1). When catastrophic cell death was considered by itself, a significant

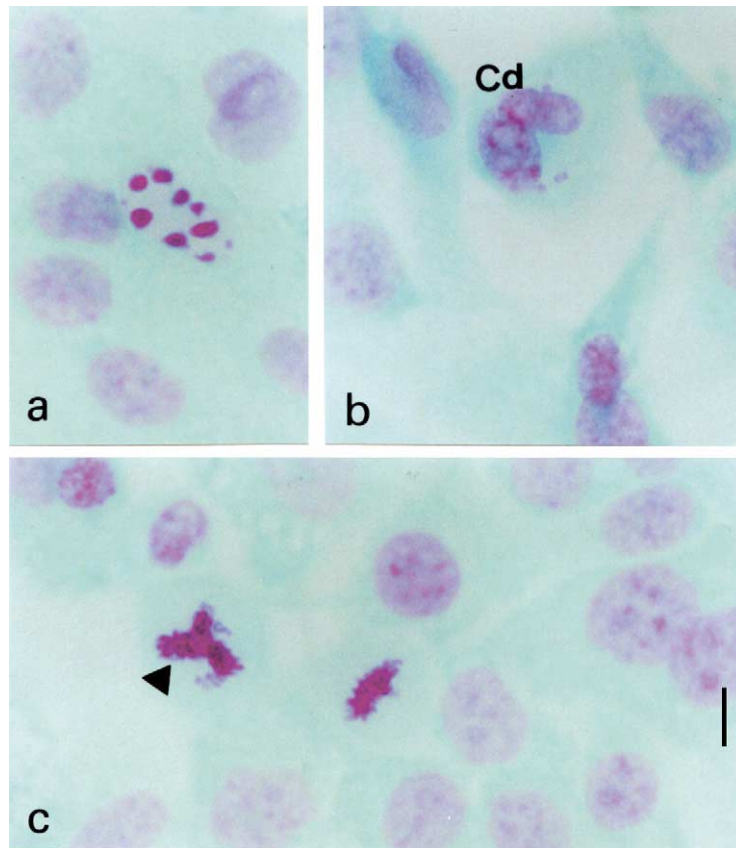


Fig. 1. Apoptosis (a), catastrophic death (b, Cd), and aberrant mitosis (c, arrowhead) in BP1-E cells subjected to the Feulgen reaction plus acid fast green staining. Bar = 10 μ m.

Table 1

Cell death and cell abnormalities in transformed human breast epithelial cells after insertion of normal chromosomes 11 and 17

Cell lines	Number of cells analyzed ^a	Apoptotic ratio (%) ^b	Catastrophic death (%) ^b	Giant mono-nucleate cells (%) ^b	Micronucleate cells (%) ^b	Abnormal mitosis (%) ^b	NIR (%)
BP1-E	7546	0.25 (0.18–0.32)	0.50 (0.40–0.59)	0.56 (0.47–0.67)	0.53 (0.36–0.68)	0.87 (0.67–1.00)	2.50
BP1E-11neo	7590	0.80* (0.65–0.87)	0.29 (0.20–0.40)	0.38 (0.32–0.48)	0.38 (0.32–0.44)	0.26* (0.20–0.31)	1.31*
BP1E-17neo	7575	1.01** (0.75–1.23)	0.12* (0.07–0.16)	0.36 (0.32–0.44)	0.17* (0.07–0.28)	0.30* (0.16–0.43)	0.96*
MCF-10F	6000	1.53 (1.30–1.90)	0.13 (0.00–0.20)	–	–	–	–

NIR = nuclear instability ratio.

^a Analysis done in triplicate.^b Minimum/maximum indices.* Significantly different from BP1-E values at $P < 0.05$, using Goodman's test [20];** Significantly different from BP1-E values at $P < 0.001$, using Goodman's test [20];

decrease was detected only when normal chromosome 17 was inserted into BP1-E cells (Table 1).

Under present in vitro cell growth conditions, the frequencies of apoptosis, catastrophic cell death and cell abnormalities were reasonably repetitive as indicated in Table 1. The results do not reflect clonal variation since more than one clone containing chromosome 11 or 17 were investigated.

4. Discussion

These results agree with the observation that in BP1-E cells the tumor suppressor sequences encoded in normal chromosomes 11 and 17 are affected by microsatellite instabilities and the loss of heterozygosity [8,9,21]. Furthermore, the differences between results provided by chromosomes 11 and 17 transfer support the assumption of a region of chromosome 17 being mostly responsible for apoptosis mediation in MCF-10F cells, since the transfer of normal chromosome 17 to BP1-E cells involves reversal of the transformed phenotype by FAS receptor activation, which is known to induce apoptosis [14]. In addition, microsatellite instability has recently been considered to be responsible for the loss of an apoptotic pathway in ductal breast carcinomas [22]. The presence of a normal chromosome 17 is necessary for tumorigenic human breast epithelial cells to recover their normality in terms of DNA content and geometric parameters [13], whereas a normal chromosome 11 contributes to the control of RNA transcript surplus production [23].

It is interesting that the introduction of normal chromosome 17 in BP1-E cells promotes apoptosis

elicitation simultaneously to decrease in frequency of catastrophic death. Maybe these cell death forms are in some way metabolically interconnected such that apoptosis elicitation blocks paths that otherwise would lead cells to catastrophic mitosis.

Some dosage effect of additional copies of specific genes on apoptosis and nuclear instability is discarded because after the transfer of chromosome 11 or 17 to non-transformed MCF-10F cells any phenotypic feature was altered (Lareef, unpublished data).

Present results indicate that the nuclear stability conferred by chromosomes 11 and 17 in non-transformed MCF-10F cells and which became affected by microsatellite instability and loss of heterozygosity with BP treatment [8,9], also involves the control of cell death mechanisms and abnormal mitosis. The introduction of normal chromosomes 11 and 17 in tumorigenic BP1-E cells by MMCT is thus a useful tool for demonstrating several cell parameters, including the mechanisms of cell death that are altered after transformation with BP.

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