

RNA relocation and persistence of nucleolus-like bodies at mitosis in benzo[a]pyrene-transformed human breast epithelial cells after microcell-mediated transfer of chromosomes 11 and 17

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RNA relocation and the incidence of nucleolus-like bodies accumulated during mitosis were studied cytochemically in benzo[a]pyrene (BP)-transformed human breast epithelial MCF-10F cells after microcell-mediated transfer of normal chromosomes 11 and 17. The changes resulting from the transfer of these two chromosomes in tumorigenic MCF-10F cells (BP1-E cell line) were examined, since alterations in these chromosomes are involved in the expression of the transformed and tumorigenic phenotypes in the MCF-10F cell series. In addition, the frequency of nucleolus-like bodies decreases drastically with transformation and tumorigenicity in MCF-10F cells, thus being conceivable that it would be affected in presence of normal chromosomes 11 or 17. The pattern of RNA relocation associated with the mitotic spindle did not vary in the cell lines analyzed. The introduction of chromosome 17 in BP1-E cells either decreased or did not affect the frequency of persistent nucleolus-like bodies. In contrast, in cells which received a normal chromosome 11, the frequency of nucleolus-like bodies was closer to that of non-transformed MCF-10F cells. These results suggest that a normal chromosome 11 but not chromosome 17 contributes to the maintenance of an RNA surplus which accumulates in nucleolus-like bodies during cell division of the human

breast epithelial cells, at least *in vitro*. Some loci which were retained in the BP1-E cells which received a normal chromosome 11 are probably involved with the control of RNA transcript production.

Figure 1 on http://www.esacp.org/acp/2001/23-3_4/mello.htm.

Keywords: RNA, nucleolus-like bodies, human breast epithelial cells, microcell-mediated chromosome transfer, chromosome 11, chromosome 17

1. Introduction

When transformed with benzo[a]pyrene (BP), human breast epithelial MCF-10F cells cultured *in vitro* produce cell lines which show gradual stages of tumoral progression (BP1, BP1-E, BP1-E1 among others) [3]. RNA relocation associated with the mitotic spindle fibers does not vary during cell division in transformed MCF-10F cell series [10], as shown cytochemically using a variant of the critical electrolyte concentration assay [8]. However, the frequency of RNA-containing nucleolus-like bodies which persist during mitosis, and which were primarily described in non-transformed MCF-10 cells and assumed to be due to a non-profitable surplus of RNA [8], decreases drastically in transformed and tumorigenic MCF-10F cells [10]. In contrast, a significant increase in nucleolar size and rRNA production in interphase nuclei occurs in the BP1-E and BP1-E1 tumorigenic cell lines relative to non-tumorigenic, transformed cell line BP1 and non-transformed MCF-10F cells [1]. These findings have been explained in terms of an improved use of RNA transcripts during cell transformation and tumorigenesis, at least under *in vitro* conditions [10].

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Microsatellite instability markers in chromosomes 11 and 17 have been associated with neoplastic progression in BP-transformed MCF-10F cells [6]. Microsatellite instability may provoke defects in DNA replication or mismatch repair mechanisms [2,12,14]. Although microsatellite instability is not so commonly found in breast tumors as in colorectal cancer [7], cases have been reported in which microsatellite instability was considered an early event during human breast carcinogenesis [15,17]. Recently, microsatellite instability has been correlated with hormonal deregulation in the progression of breast cancer [4] and the cause of loss of an apoptotic pathway in ductal breast carcinomas [13]. Nearly 20% of microsatellite markers have been revealed in 33% of patients with primary breast tumors studied by Wild and co-workers [18], provided small tumor areas were removed by precise laser-microdissection. There are also reports indicating that in 11.9% of sporadic breast cancers, microsatellite instability was correlated with more advanced disease and relatively poorer prognosis [16].

When normal chromosomes 11 and 17 were transferred to tumorigenic BP1-E cells by microcell-mediated chromosome transfer (MMCT) assays, a functional role of these chromosomes in the expression of the BP1-E transformed phenotypes was demonstrated [19]. Some of the tumorigenic BP1-E cell characteristics which reverted to those of non-transformed MCF-10F cells by transfer of normal chromosomes 11 and 17 were: decrease in cell growth, reduced colony efficiency and colony size [19], and a tendency to restoration of the DNA amount and nuclear sizes to the distribution patterns typical of the non-transformed cells [11]. Telomerase activity was significantly reduced by chromosome 17 insertion [19]. It is thus conceivable that the pattern of RNA distribution and of the incidence of nucleolus-like bodies during mitosis under the same above-cited experimental conditions may also be affected. In the present study, RNA relocation and the incidence of nucleolus-like bodies during cell division were studied cytochemically in tumorigenic, BP-transformed MCF-10F cells receiving normal chromosomes 11 and 17 through MMCT assays.

2. Materials and methods

2.1. Cells

BP1-E, a BP-transformed cell line derived from MCF-10F cells currently maintained in the Breast

Cancer Research Laboratory of the Fox Chase Cancer Center in Philadelphia as reported previously [3], was used in the transfer of normal chromosomes 11 and 17 by microcell-mediated chromosome transfer (MMCT). BP1-E 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 $\mu\text{g/ml}$) were subcloned. Four expandable clones (subclones) containing normal chromosomes 11 and 17 were used. BP1-E cells at passage 45, and BP1E-11neo and BP1E-17neo cells at passage 7 were used. The cells were grown for 48 h (BP1-E, BP1E-11neo and BP1E-17neo) or 96 h (BP1E-17neo) on well slides and fixed. Since BP1-E cells and the clone with transferred chromosome 11 grew faster than the clone transferred with chromosome 17, a confluence value of 80% was attained by these cells at different growth times (BP1-E and BP1E-11neo, 48 h; BP1E-17neo, 96 h).

2.2. Cell preparations and staining procedure

The cells were fixed in an ethanol-acetic acid mixture (3:1, v/v) for 1 min, rinsed in 70% ethanol for 3–5 min, and air dried before the identification of RNA by a variant of the critical electrolyte concentration (CEC) assay, which used toluidine blue (TB) and Mg^{2+} ions as competitors for the substrate binding sites. At the DNA CEC point, DNA metachromasy (violet colour) is abolished, whereas RNA metachromasy remains unchanged ($\text{CEC}_{\text{RNA}} > \text{CEC}_{\text{DNA}}$) [9]. Briefly, the cells were stained with 0.025% TB (Merck) solution in McIlvaine buffer at pH 4.1 for 15 min and then treated with a 0.05 M aqueous solution of MgCl_2 for 15 min. The slides were then rinsed in distilled water, air-dried, cleared in xylene and mounted in Canada balsam [9]. Preparations treated with a 0.01% RNase III (Sigma) aqueous solution for 1 h at 37°C prior to the CEC assay were used as controls. The slides were examined and photomicrographed with a Zeiss Axiophot II microscope.

2.3. Mitosis counting

Three slides corresponding to six wells of each cell line (or clone) were examined for mitotic cells. Nearly 70 dividing cells were chosen randomly from each

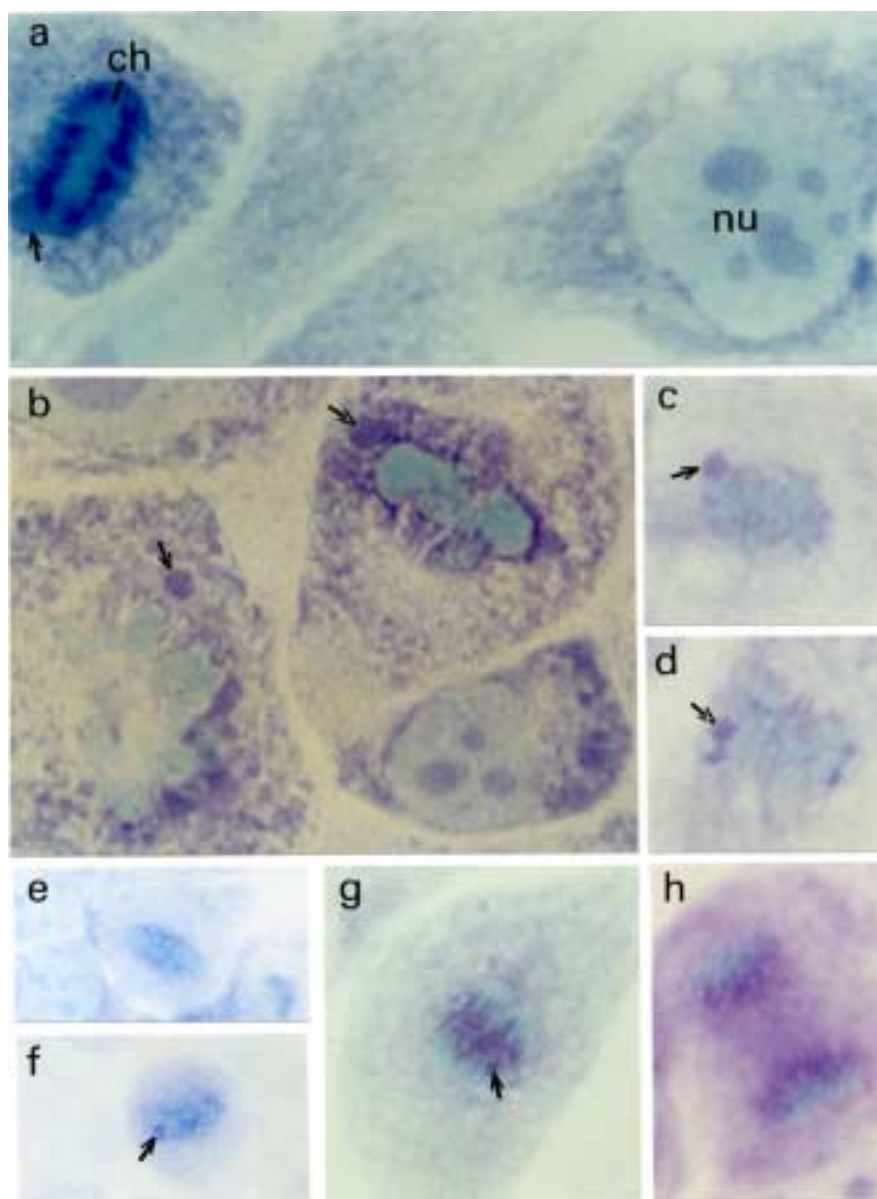


Fig. 1. Metachromatic staining of interphase cell nucleoli (nu) (a, b), nucleolus-like bodies (arrows) (a-d, f, g), and RNA along the mitotic spindle (a, g, h) in MCF-10F cell lines. e, h: cells devoid of conspicuous nucleolus-like bodies; ch: chromosomes. a-d, g, h, $\times 1800$; e, f, $\times 350$. This figure can be viewed on http://www.esacp.org/acp/2001/23-3_4/mello.htm.

slide and classified based on absence or presence of nucleolus-like bodies. In the case of BP1E-17neo cells grown for 48 h, the number of dividing cells analyzed per well was 25.

3. Results

RNA was identified by its metachromatic staining which could be prevented by pretreatment with

RNase [9]. This staining was concentrated in the nucleoli of interphase cell nuclei (Fig. 1a, b) or lined the chromosomal mass which migrated to the equatorial plate of mitotic cells (Fig. 1a, b, e, f) and then interspaced the sets of chromosomes moving to the cell poles during anaphase/telophase (Fig. 1g, h). The pattern of RNA relocation associated with the mitotic spindle was the same in all cells examined and agreed with previous data [10].

Table 1
Incidence of nucleolus-like bodies

Cells	Cell culture		Metaphases	
	Growth (h)	Confluence (%)	No. of cells counted	Nucleolus-like bodies frequency (%)
BP1-E	48	80	218	16
BP1E-11neo	48	80	221	74
BP1E-17neo	48	50	150	6.7
	96	80	246	19.5

When nucleolus-like metachromatic bodies were present, they appeared close to the chromosomes or to the mitotic spindle in dividing cells (Fig. 1a–d, f, g). The frequency of these bodies in mitotic cells of the various cell lines is shown in Table 1. This frequency was low in BP1-E cells which received a normal chromosome 17, even when the level of culture confluence was the same as that of cultured BP1-E cells with transferred normal chromosome 11. In cells which received a normal chromosome 11, the frequency of nucleolus-like bodies was closer to that of non-transformed MCF-10F cells (86%) [10].

4. Discussion

Figure 1 can be viewed on http://www.esacp.org/acp/2001/23-3_4/mello.htm.

The transfer of normal chromosomes 11 and 17 into tumorigenic BP1-E cells has been reported to result in a 50% and 90% inhibition of cell growth, respectively, and to reduce both colony efficiency and colony size [19]. Additionally, it has been found to increase cell population DNA amounts and nuclear sizes to values typical of non-transformed MCF-10F cells [11]. In terms of the nucleolus-like bodies seen during cell division in BP1-E cells, present data indicate that the frequency of these bodies increases in presence of a normal chromosome 11, becoming closer to that of non-transformed MCF-10F cells [10]. It is thus assumed that specific regions of chromosome 11 play a functional role in the expression of phenotypes characteristic of tumoral progression in human breast epithelial cells *in vitro*, including those related to the control of RNA transcript production which is probably concerned with the accumulation of an RNA surplus during cell division.

Microsatellite polymorphism analysis has revealed that the loci 11q13–23, 11q23.1, 11q23.3, and 11q25 are retained in the BP1-E cells which receive a normal chromosome 11 through MMCT techniques [19].

Data from human genome maps in the web [5] reveal that the splicing factor 3b, subunit 2, 145 kD (SF3B2), a spliceosome-associated protein which participates in anchoring of U2 snRNP to pre-mRNA, the RNA binding motif protein 14, the breast cancer metastasis-suppressor 1 (BRMS1), and the tumor suppressor 14 (ST14) are encoded in these regions and possibly related to the above-mentioned characteristics of the BP1-E cells with transferred normal chromosome 11.

The higher frequency of nucleolus-like bodies in BP1E cells which received a normal chromosome 11 may reflect a certain poorly controlled RNA transcript production *in vitro*. This would lead to an RNA surplus accumulation during cell division in a response similar to that in non-transformed MCF-10F cells [10]. With the gradual advancing of tumorigenesis in transformed MCF-10F cells, alterations in chromosome 11 may lead cells to improve their use of RNA transcripts.

The unchanged pattern of the RNA relocation in association with the mitotic spindle architecture during cell division under present experimental conditions indicates that this process does not require normal chromosomes 11 or 17 to be effective.

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