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Tumor Cell Fusion and Multipolar Trivision

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Abstract

The fusogenic character of tumor cells has been suspected, without direct visualization of such processes. Fusion is likely to create polyploid cells, instability, chromosome loss, and induce tumorigenicity. The aim of this study was to provide direct evidence for the fusion and trivision of hyperploid tumor cells and their viability. Cell fusion was captured by time-lapse microscopy in hypertriploid (> 3N) HeLa cell culture followed by trivisions and seen with relatively high frequency (1:24, 4%) relative to cell divisions. The diameters of round M phase HeLa, HaCaT and uveal melanoma cells and their cell volumes before trivision were significantly larger than the size of cells to be divided, while the average volumes of trivided cells were smaller than divided cells. The volume of trivided smaller cells was not uniform indicating an uneven distribution of cytoplasmic material. We provide cinemicrophotographic evidence that after fusion the hybrid HeLa cell undergoes trivision and generates one large and two smaller cells. In the next cell cycle the larger daughter cell divides in two cells, the two smaller daughter cells fuse and trivide. Fused hybrid tumor cells are viable and generate further trivisions and divisions conforming to the cell fusion hypothesis suggesting that fusion generates aneuploid small tumor cells that can lead to metastasis.

Keywords: Hyperploids cells; Hybrid tumor cells; Aneuploidy; Rediploidization; Cinemicrophotography

Introduction

Cell fusion plays a key role in early embrional development [1-4]. Artificially fused myeloma cells with lymphocytes known as hybridomas produce monoclonal antibodies. Hybridized cells can be implicated in the progression of cancer [5,6], and in the diversity of tumors [7]. Cell fusion may cause cancer and lead to metastasis, but this hypothesis never gained sufficient recognition [8]. The rarity of fusion, the scarcity of reports and the difficulty of detection could explain why cell fusion remained poorly understood [9]. The fusion of mammalian cells into syncytia is regarded as a developmental process that is tightly restricted to a limited subset of cells [10]. HeLa cells expressing fusion proteins have been used to analyse glycoproteins in virus-induced cell fusion, leading to the conclusion that the expression of the fusion protein F enhances susceptibility to cell fusion [11].

Although, the fusion of tumor cells is a rare event, but could be captured several times during the more than 150 time-lapse image analyses performed. The occurrence of cell trivisions relative to divisions was the highest in hyperploid HeLa cells (1:24, 4%), followed by HaCaT (1:126, 0.8%), and uveal melanoma cells (1:186, 0.5%). The frequency of trivision was much lower in near diploid endothelial cells (1:1400, 0.07%) [12]. Due to the slower movement and stronger adherence, HeLa cells remained for a longer period of time in the visual field during time-lapse microscopy than other cells allowing the visualization of the same cells for two consecutive trivisions.

Results and Discussion

Increased size of cells to be trivided: After trivision the diameter of the larger progenitor cell (1a = 16.60 ± 0.68μm) differed significantly from the other two smaller cells (1b = 12.91 ± 0.78μm and 1c = 12.94 ± 0.40μm) and from the average size of HeLa cells (diameter 16.93 ± 1.11μm, volume 2540 ± 594 fl). Similarly, the average diameter of the trivided cells was smaller than the average HaCaT cell (24.47 ± 1.34μm) with three smaller daughter cells in HaCaT (1a = 20.19 ± 0.48 μm, 1b = 19.19 ± 1.02μm, 1c = 18.40 ± 0.37μm), one larger daughter cell in uveal melanoma cells (1a =17.92 ± 0.29μm) and two smaller daughter cells (1b = 12.9 ± 0.35μm, 1c = 12.20 ± 0.40μm) relative to divided uveal melanoma cells (17.11 ± 1.65μm) (Figure. 1).

Table 1: Duration of trivisions relative to divisions

	HaCaT	UM	HeLa
Division (min)	11.6 ± 1.44 (n=10)	31.9 ± 5.50 (n=10)	22.3 ± 5.36 (n=5)
Trivision (min)	45.5 ± 1.0 (n=10, ** = p < 0.01)	43.9 ± 13.46 (n=10, p > 0.05)	42.8 ± 6.24 (n=5, ** = p < 0.01)

In the three series of experiments (HaCaT, UM, HeLa) each set of trivision time was tested relative to its own appropriate division control. Time-lapse microscopy: n = 10 for HaCaT and uveal melanoma (20 divided and 30 trivided cells), n=5 for HeLa (10 divided and 15 trivided) cells. Statistical analysis: * = p < 0.05, ** = p < 0.01.

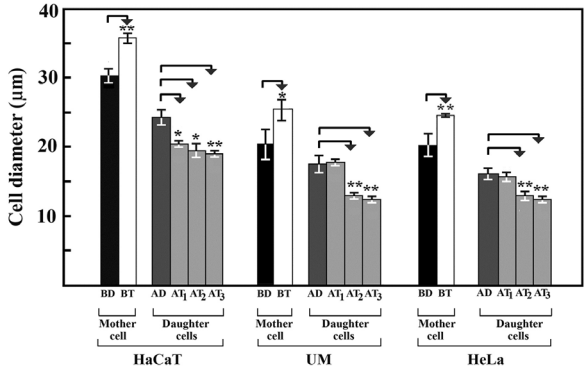

Figure 1: Cell diameter before and after cell division and trivision. HaCaT, uveal melanoma (UM) and HeLa cells were subjected to time-lapse image microscopy. The diameter of mitotic cells was measured after their detachment and rounding up. Abbreviations: BD, before division; BT, before trivision; AD, after division; AT, after trivision; AT1, AT2, AT3, trivided daughter cells. In the three series of experiments (HaCaT, UM, HeLa) each set was tested relative to its own appropriate BD and AD control. Time-lapse microscopy: n = 10 for HaCaT and uveal melanoma (20 divided and 30 trivided cells), n = 5 for HeLa (10 divided and 15 trivided) cells. Statistical analysis: * = p < 0.05, ** = p < 0.01.

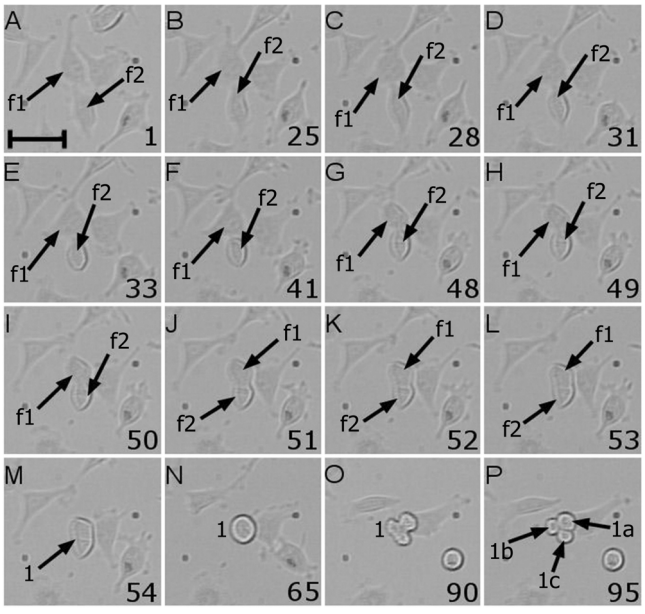

Figure 2: Cell fusion and first cell trivision of fused HeLa cell. Photographs of growing cells were taken every min by our custom-built video camera attached to the microscope and connected to the computer. A-H: Cells (f1 and f2) before fusion. I-L: Fusion of f1 and f2 cells. M-N: fused cell indicated by number 1. O-P: Trivision of cell #1 to 1a, 1b, 1c cells. As orientation black arrows point to the cells undergoing fusion, to the fused cell and to the trivided cells. Black number at the bottom of each frame shows the time of photography. Scale bar: 50 µm for each frame. Exposures were converted to videofilm by speeding up the projection to 25 exposures/s

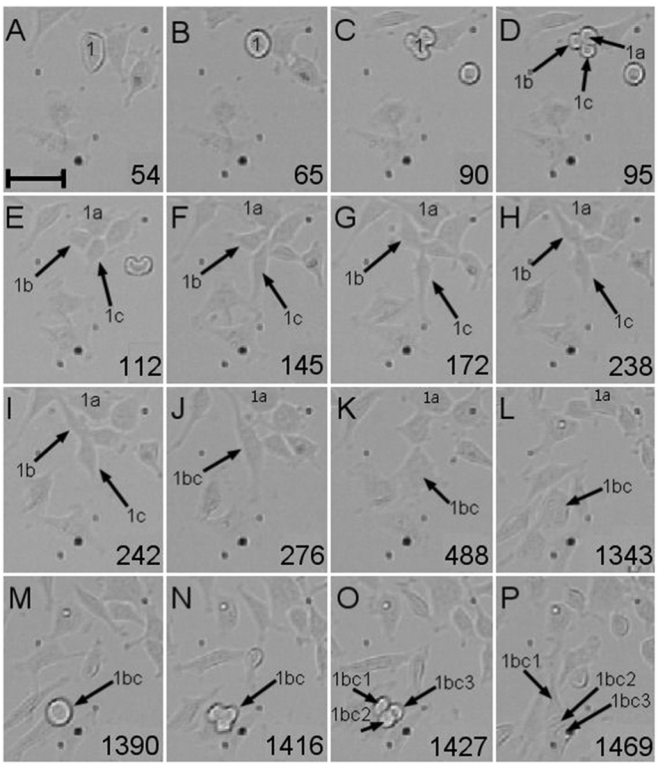

Figure 3: Time-lapse photography of fused HeLa cell undergoing second trivision. The same montage of time-lapse microscopy is presented an in Figure. 2. A-D) First trivision. E-I) Growth of trivided daughter cells. I-J) Fusion of the two smaller (1b, 1c) trivided cells. K-L) Growth of fused 1bc cell. M-O) Trivision of the fused 1bc cells. P) Growth of the tree daughter cells of the second trivision. Black arrows show the position of the fusion and the second trivision of the fused cell. Black number at the bottom of each frame indicates the time of photography. Scale bar: 50 µm, each frame.

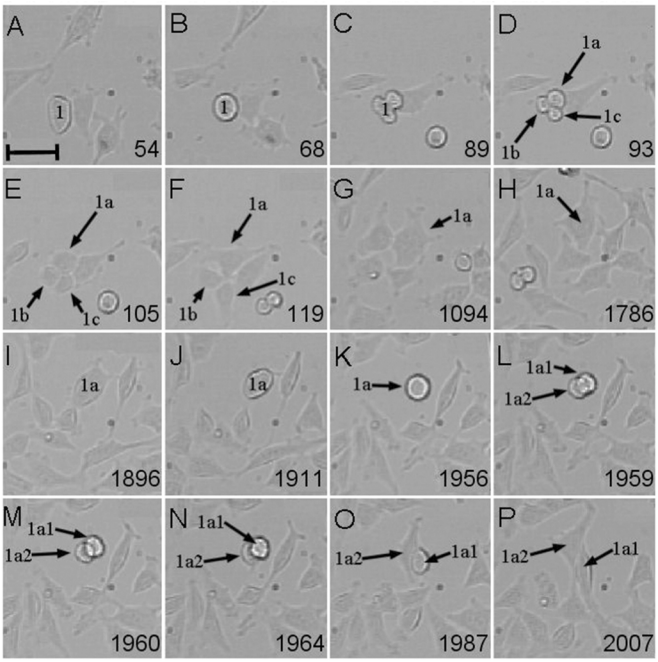

Figure 4: Time-lapse photography of HeLa cell undergoing trivision followed by division of its large daughter cell. A-F) Trivision (1a, 1b, 1c). G-P) Division of the larger daughter cell (1a) of trivision. Labels are the same as in Figure. 1. Scale bar: 50µm.

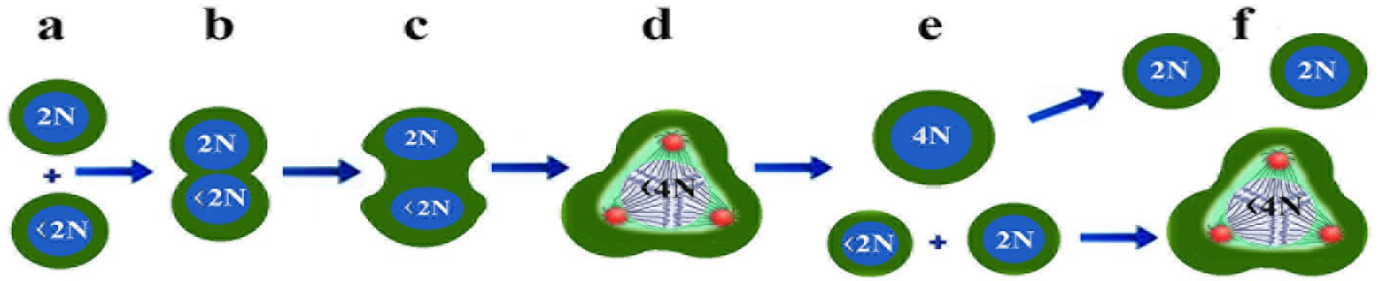

Figure 5: Schematic view of cell fusion and multipolar cell trivision in HeLa cells. Two cells in the cell culture (at least one of them hypodiploid) (a), attach (b), merge (c) and undergo complete cell fusion (<4N) (d). Fusion is followed by growth (<8N), first cell trivision, forming multipolar cells, one large (4N) and two small cells (2N and <2N) (e). Large cell (4N) undergoes normal cell division (2N + 2N). The fusion of the two smaller cells leads to second trivision (f).

Comparison of division and trivision times: During the time-lapse image analysis the time of division or trivision was defined as that period of time expressed in minutes that lasted from the rounding up of mother cell to the attachment of daughter cells. Statistical analysis of time-lapse videomicroscopy indicates that the duration of trivision lasted significantly longer in HaCaT and HeLa cells (Table 1). Uveal melanoma cells showed a similar tendency, but due to the fluctuations of duration the exact time of trivision could not be exactly determined (Table 1).

Fusion of HeLa cells before first trivision: Time-lapse microscopy shows the fusion of two (f1 and f2) HeLa cells (Figure. 2A-C), with *f2* cell approaching mitosis earlier than *f1* cell seen by their rounding up (Figure. 2D and 2F). The *f1* cell started to round up 17 min later than *f2* cell (Figure. 2G). The rapid fusion of cells took about 5 min (Figure 2H-2M). The trivision of the fused cell lasted for approximately 36 min (Figure. 2N - 2P).

Fusion and second trivision of small progenitor cells of the first trivision: Figure. 3 is the same as Figure. 2, with scrutiny placed on the fate of the two smaller (1b and 1c) daughter HeLa cells of the first trivision (Figure. 3D). These two smaller cells grew separately for 2h 27min (Figure. 3D–3I), and fused 34 min later to produce one large (1bc) cell (Figure. 3I-3J). The generation time between the two consecutive trivisions (22 hour 5 min) calculated with the aid of cinephotomicroscopy from the appearance of the round mitotic cells before the first and second trivision (time of Figure. 3M minus 3B) shows that the cell cycle of trivision is longer than the regular cell cycle of division (14-16h) of HeLa cells. Cells of the second trivision (1bc1, 1bc2 and 1bc3) settled down and continued their growth, prowing their viability (Figure. 3P).

Division of large daughter cell of the first trivision: Figure 4 follows the fate of the large daughter cell (1a) after the first cell trivision (Figure. 4D). Growth of this large (1a) daughter cell is seen in Figure. 4G-4P. Division of 1a cell took place 31h 28min after trivision calculated from the appearance of round mitotic cells (Figure. 4K minus 4B) generating two cells of nearly equal size (Figure. 4L-4N). Divided 1a1 and 1a2 daughter cells settled down and continued their growth (Figure. 3O-3P), prowing that not only trivided by but also divided cells upon cell fusion are viable.

Our results indicate that cell fusion, similarly to malignant transformation increases the ploidy and tumor cells may become tetraploid [13]. Tumor cells try to avoid the metastable state between normal euploidy and cancer-associated aneuploidy, and return to diploidy resulting in a near diploid, yet aneuploid state [14-16]. One of the mechanisms of polyploidy of tumor cells is cell fusion beside endoreplication, cytokinesis failure and cannibalism by entosis generating nearly tetraploidy (~4N). As far as the resolution of the tetraploid state is concerned two mechanisms have been proposed: a) after the merothelic attachment the cell might divide in a bipolar fashion producing two larger cells with 4N+1 and 4N-1 aneuploidy, and b) by tripolar mitosis the nearly tetraploid cells can give rise to gross aneuploidy of three daughter cells with different size and DNA content (xN, yN, zN) [17]. Alternatively, during tripolar division sister chromatid separation may proceed in a regular fashion, yet cytokinesis failure turns to an asymmetrical segregation and chromosomes are divided in two daughter cells, one of them carrying and extra chromosome copy generating trisomy [18]. After tripolar mitosis cells do not aquire proper diploid chromosome number and are constant sources of genomic instability. A further outcome, namely that polyploid cells might be arrested without cytokinesis and undergo cell death can be excluded as apoptosis was not seen during trivision.

The results obtained by video time-lapse microscopy favour the theory of tripolar mitosis and solve the enigma of how human cancer cells bypass the metastable hyperploid state. Re- diploidization of hyperploid genomes manifested as cell trivision decreases the average size of daughter cells [19] with one larger and two smaller cells than the average cell size. Trivision is preceeded by the fusion of two cells, at least one of them being an aneuploid tumor cell. The larger daughter cell of trivision seems to undergo regular division. These observations provide a reasonable base for the speculation that smaller aneuploid cells are more dangerous and probably accountable for the maintainance of the hyperploid precancerous state of polyploidy.

Conclusion

The major steps of cell fusion followed by tripolar mitosis are schematically viewed in Figure. 5. The spontaneous fusion of two cells (Figure. 5a) involves membrane (Figure. 5b), cytoplasmic (Figure. 5c) and nuclear fusion (Figure. 5d). The polyploid state of the fused cell is resolved by tripolar mitosis generating three daughter cells (Figure. 5e) and lead to further trivision (Figure. 5f) in agreement with the theory of multipolar mitosis [17]. Abnormal, tripolar mitosis can be the result of genotoxic treatment such as mustard gas [20] or extended

colcemid block [21]. Based on the results we hypothesize that a) hyperploidy represents an intermediate stage between normal diploidy and aneuploidy, b) cell trivision induces random aneuploidy by producing one nearly tetraploid cell that undergoes normal division and two smaller cells, with at least one of them being hypodiploid, c) aneuploidy is likely to generate new cancer-specific karyotypes, d) the rate of cancer development is proportional to the frequency of fusions, trivisions and aneuploidy, e) cell fusion and coupled trivision in hyperploids tumor cells are likely causes of small cell tumorigenesis. Consequently, the induction of hyperploidy cannot be used as a therapeutic strategy to force cells into apoptosis.

Materials and Methods

The growth of individual cells can been traced by video microscopy up to several days [22]. However, the genotoxic effect could last longer [23] than cinephotomicroscopy could follow and fluorescence microscopy may cause phototoxicity [24]. A further shortcoming of cinemicroscopy was that cells outside CO_2 incubators grew under nonphysiological conditions. These technical hindrances have been overcome by avoiding photo- and chemotoxicity using charge-coupled device cameras connected to the computer and replacing old medium with fresh one using peristaltic pumps and extending observations for an unlimited period of time inside the CO_2 incubator [25].

To study cell trivision we used hyperploid tumor cells that perform cell fusion with greater frequency. Time-lapse microscopy was performed with four rather than two synchronously working inverted microscopes. Further precautions served the prevention of artificial morphological transitions to get reproducible results [12]. Beside cell fusion, endoreplication, cytokinesis failure and cannibalism by entosis are potential causes of hyperploidy [17]. We provide evidence by the time-lapse micrography that the fusion of two smaller aneuploid HeLa cells is followed by tripolar mitosis and could be responsible for the maintenance of aneuploidy in the cell culture.

The description of HeLa (ATCC CCL-2), HaCaT, uveal melanoma cell lines, diploid bovine artery endothelial cell culture (ATCC CCL 209) and their growth in Dulbecco's modified Eagle's medium (DMEM) is given elsewhere [12]. The time-lapse microscopy system was described earlier [12,19]. The fusion of cells was taken as initial frames of the photography and the reattachment of daughter cells of the second trivision as finish.

Competing interest

The authors declare that they have no competing interests.

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