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Endopolyploidy in irradiated p53-deficient tumour cell lines: Persistence of cell division activity in giant cells expressing Aurora-B kinase

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Abstract

Recent findings including computerised live imaging suggest that polyploidy cells transiently emerging after severe genotoxic stress (and named 'endopolyploid cells') may have a role in tumour regrowth after anti-cancer treatment. Until now, mostly the factors enabling metaphase were studied in them. Here we investigate the mitotic activities and the role of Aurora-B, in view of potential depolyploidisation of these cells, because Aurora-B kinase is responsible for coordination and completion of mitosis. We observed that endopolyploid giant cells are formed via different means in irradiated p53 tumours, by: (1) division/fusion of daughter cells creating early multi-nucleated cells; (2) asynchronous division/fusion of sub-nuclei of these multi-nucleated cells; (3) a series of polyploidising mitoses reverting replicative interphase from aborted metaphase and forming giant cells with a single nucleus; (4) micronucleation of arrested metaphases enclosing genome fragments; or (5) incomplete division in the multi-polar mitoses forming late multi-nucleated giant cells. We also observed that these activities can release para-diploid cells, although infrequently. While apoptosis typically occurs after a substantial delay in these cells, we also found that ~2% of the endopolyploid cells evade apoptosis and senescence arrest and continue some form of mitotic activity. We describe here that catalytically active Aurora-B kinase is expressed in the nuclei of many endopolyploid cells in interphase, as well as being present at the centromeres, mitotic spindle and cleavage furrow during their attempted mitotes. The totally micronucleated giant cells (containing sub-genomic fragments in multiple micronuclei) represented only the minor fraction which failed to undergo mitosis, and Aurora-B was absent from it. These observations suggest that most endopolyploid tumour cells are not reproductively inert and that Aurora-B may contribute to the establishment of resistant tumours post-irradiation.

Keywords: Mitotic catastrophe; Tumours; Polyploidy; Aurora-B kinase

1. Introduction

The application of genotoxic insults including irradiation is an established method of treatment of malignant tumours. Tumours lacking functional p53 are defective in many cell cycle checkpoints and often respond to genotoxic stress by undergoing mitotic catastrophe (MC). Although MC is defined as ''cell death occurring during or shortly after a failed mitosis'' (Kroemer et al., 2005; Galluzzi et al., 2007), p53-deficient tumours undergoing MC are resistant to genotoxic treatments. As a result of mitotic failure, cells alternatively reset interphase becoming tetraploid (Castedo et al., 2004a,b). Therefore, MC has also been defined as mitotic events that produce tetraploid progeny cells in the first post-damage

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generation (Andreassen et al., 2001). p53 mutant tumour cells that have incurred genotoxic stress and become tetraploid can continue endoreplication and achieve DNA content from 8C to 64C (Illidge et al., 2000; Chu et al., 2004). Association of genotoxic resistance with the induced endopolyploidy was found in rodent and human tumours (Baroja et al., 1998; Come et al., 1999). Our earlier observations revealed that transient endopolyploid p53-Burkitt lymphoma cells were able to facilitate DNA repair and release para-diploid mitotic progeny post-irradiation (Erenpreisa et al., 2000; Illidge et al., 2000; Ivanov et al., 2003). These observations led us to hypothesise that transient endopolyploid cells, which are capable of depolyploidisation, may in fact constitute an alternative survival pathway (Erenpreisa and Cragg, 2001, 2007). Similarly, de la Hoz and Baroja (1993) and Baroja et al. (1996, 1998) reported that rodent tumour cells of high ploidy are capable of proliferating, despite certain peculiarities in their cell cycle. Using computerised live imaging, Ianzini and Mackey (2002) have demonstrated that a small proportion of endopolyploid cells formed *in vitro* post-mitotic catastrophe successfully undergo polyploidy reduction and form viable clones. Prieur-Carrillo et al. (2003) found that ~2% of human bladder carcinoma giant cells formed after irradiation release potentially clonogenic 2N progeny. Stewenius et al. (2005) showed that events of mitotic catastrophe in colorectal cancer are compatible with survival, and underlined the role of anaphase bridged mitoses in clonogenic growth. Furthermore, the striking live-imaging studies of Chu et al. (2004) on CDKN1A-deficient cells (CDKN1A is up-regulated by the tumour suppressor p53 controlling G1/S checkpoint) have clearly shown the viability of the endopolyploid cells produced by multiple mitotic catastrophe events. These authors concluded that MC is not directly responsible for individual cell death. Similar observations were made and reviewed by Rajaraman et al. (2006).

These intriguing reports underscore the importance to study further the division potential of endopolyploid cells in p53-deficient tumours. Although the presence of high ploidy cells in malignant tumours has long been documented (Baroja et al., 1998), their biological significance is not well understood, with much controversy persisting over their proliferative potential. However, if as a result of genotoxic treatment, genetically unstable giant cells can give rise even to a few selected clones, these might be genetically changed, promoting resistant regrowth and further tumour progression. Therefore detailed study of the mechanisms of the reproductive/apoptotic behaviour of giant cells is important.

We have investigated the reproductive activities of endopolyploid cells post-irradiation in p53 defective human cell lines through the involvement of Aurora-B kinase, the essential regulator of mitosis (Carmena and Earnshaw, 2003; Vagnarelli and Earnshaw, 2004). Aurora-B belongs to the group of mitosis regulators called "chromosome passengers". Within this group, Aurora-B kinase provides for fidelity and procession of mitosis by coordinating chromosome alignment onto metaphase spindle with anaphase and cytotomy (Ditchfield et al., 2003). The easily recognisable immunocytochemical markers of its presence are the attachment of Aurora-B to centromeres in metaphase plate, to microtubules of the central mitotic spindle during anaphase B and participation in the formation of the mid-body in ana-telophase. The mid-body is marked by the two bands of Aurora-B and two lateral bands of tubulin. In immunofluorescent staining for the two proteins, these two-coloured bands and a central split in the mature mid-body (the place of the centriolin ring) assign the whole structure its unique appearance. While the main events of mitosis occur within 1 h, the mid-body, which is responsible for cytotomy completion, persists in the cytoplasmic bridge between daughter cells for 2+ hours longer (Gromley et al., 2005). Thus the mid-body represents a characteristic marker of the process of mitosis. Our data reveal that catalytically active Aurora-B kinase is intimately associated with the formation, division, and extended survival of endopolyploid cells resulting from MC in functionally p53-deficient tumour cell lines.

2. Methods

2.1. Cell lines

Namalwa Burkitt's lymphoma cells (ATCC) were grown as suspension cultures in RPMI 1640 medium, 10% foetal calf serum (Gibco or Sigma) at 37 °C in a 5% CO_2 in air humidified incubator. HeLa S3 cells (ATCC) were grown either in suspension culture or as adherent clone 3. Suspension HeLa culture was grown under constant rotation in Joklik's MEM media containing 10% heat-inactivated calf serum (Hyclone) and antibiotics. Suspension cultures were maintained in log phase of growth for at least 24 h prior to irradiation. Namalwa cells were further cultivated by replenishing culture medium every 48–72 h, and HeLa S3 every 24 h.

HeLa adherent clone 3 cells were grown as monolayer in F-10 medium (Hyclone) containing 10% heat-inactivated foetal calf serum (Sigma or Hyclone) and antibiotics (100× penicillin–streptomycin, Sigma P4333) in a 37 °C incubator supplied with 5% CO_2 in air, either on 13 mm polylysine-coated coverglasses in 24× wells, for immunocytochemistry and DNA cytometry or in a T-25 tissue culture flasks for live-imaging.

To determine the cells in S-phase, BrdU was added at 10 μg/ml to the cell culture for 60 min prior to cell fixation on slides with methanol. DNA denaturation was performed by 2 N HCl, 37 °C for 20 min. After washes in PBS, the primary and secondary antibodies were applied (Table 1). In some experiments, proteasome inhibitors (Sigma) Mg-132 (5 μg/ml), inhibitor of calpain (25 μg/ml), and lactocystin (10 μg/ml) were added for 2 h prior to cell harvest. Autophagic vacuoles were detected by monodansylcadaverin (MDC) and by Sa-β-galactosidase. For MDC (Sigma) staining the cultures were incubated with 0.05 mM MDC at 37 °C for 60 min followed by fixation in 4% paraformaldehyde, washing twice in PBS. The slides were mounted into Perm Mount and immediately scored in the DAPI channel. For Sa-β-galactosidase detection, the instruction of the Sigma kit (Code CS0030) was followed (staining was extended overnight).