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Endoreduplication induced in cultured Chinese hamster cells by different anti-topoisomerase II chemicals Evidence for the essential contribution of the enzyme to chromosome segregation

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Abstract

With the ultimate purpose of testing the hypothesis that, as shown in yeast mutants, any malfunction of DNA topoisomerase II might result in aberrant mitosis due to defective chromosome segregation, we have chosen three chemicals of different nature, recently reported to catalytically inhibit the enzyme. The endpoint selected to assess any negative effect on the ability of topoisomerase II to properly carry out decatenation of fully replicated chromosomes in the G2/M phase of the cell cycle was the presence of metaphases showing diplochromosomes as a result of endoreduplication, i.e. two successive rounds of DNA replication without intervening mitosis. The anti-topoisomerase drugs selected were the anthracycline antibiotic and antineoplastic agent aclarubicin, the respiratory venom sodium azide, and 9-aminoacridine, a chemical compound with planar topology capable of intercalation between DNA bases. Our results show that the three chemicals tested are able to induce endoreduplication to different degrees. These observations seem to lend support to the proposal that topoisomerase II plays a central role in chromosome segregation in mammalian cells.

Keywords: DNA topo II; Endoreduplication; Aclarubicin; Sodium azide; 9-Aminoacridine

1. Introduction

The phenomenon of endoreduplication, which is rather common in plants [1], but only rarely observed to occur spontaneously in animals, has drawn a lot of attention from both cytogeneticists and investigators

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of cell-cycle genetics and biochemistry. In spite of this, no clear or unique mechanism of induction of endoreduplication has been proposed, which is partly due to the variety of agents able to induce it, but also to the various cell types in which it has been described so far. Diplochromosomes, which are the visible mitotic manifestation of this striking phenomenon, are made up of four chromatids held together, instead of the normal two, as a result of the occurrence of two successive rounds of DNA replication without intervening mitosis, i.e. segregation of daughter chromatids [2,3].

Focusing on cell-cycle stages susceptible to induction of endoreduplication by chemicals, the G2-mitosis phase appears to be the most sensitive [4–6]. Recently, in human fibrosarcoma cells, it has been reported that $p21^{waf1/Cip1/Sdi1}$-induced growth arrest is associated with depletion of mitosis-control proteins, leading to abnormal mitosis and endoreduplication in recovering cells [7]. This observation seems to be consistent with the role of the cyclin-dependent kinase (CDK) inhibitor p21 as an integral part of cell growth arrest associated with DNA damage, which in turn often involves the triggering of endoreduplication. One of the essential mammalian proteins whose expression might be inhibited by p21 is likely to be topoisomerase II (topo II), which plays a central role in chromosome segregation [8]. In mammalian cells, the existence of a temporary G2 topo II-dependent checkpoint that regulates entry into mitosis has been proposed [9].

Yeast temperature-sensitive mutants of DNA topo II fail to carry out both chromosome condensation and anaphase chromatid segregation [10–12]. In mammalian cells, on the other hand, even though the lack of available mutants represents a drawback to test the importance of the enzyme for chromosome segregation, it has been shown that topo II poisoning [13] or catalytic inhibition [14] prevents chromosome segregation and can result in endoreduplication [3].

Supporting the importance of topo II for chromosome segregation at mitosis, we have recently reported on a high yield of endoreduplication induced by the bisdioxopiperazine ICRF-193, a topo II catalytic inhibitor [15]. On the other hand, we have also recently found that endoreduplication is readily induced in AA8 Chinese hamster cells treated for two consecutive cell cycles with different halogenated nucleosides, namely 5-chlorodeoxyuridine (CldU), 5-iododeoxyuridine (IdU), and 5-bromodeoxyuridine (BrdU). Interestingly however, treatment for just one cell cycle did not lead to a similar increase in endoreduplication [16], most likely pointing to a major relative importance of template DNA as compared to the nascent molecule for proper chromosome segregation.

As regards to the specificity of the endoreduplication assay, taking into account the data mentioned above, we have recently elaborated a comprehensive model for induced endoreduplication [17], which supports the idea of the importance of inhibition of topo II function. In the present investigation, we have tested the ability of three drugs, reported to be capable of acting against topo II through different mechanisms, to induce endoreduplication: the topo II catalytic inhibitor aclarubicin [18], the cellular ATPase poison sodium azide, also considered as a catalytic inhibitor of the enzyme [19,20], and the planar DNA-intercalative drug 9-aminoacridine [21]. The results show that all three drugs are cytotoxic, show a clear inhibitory effect on topo II catalytic activity, and are able to induce endoreduplication to different degrees. These positive results, which seem to support the importance of fully operative topo II for chromosome segregation, are discussed.

2. Materials and methods

2.1. Culture conditions

The parental cell line AA8 (purchased from the American Type Culture Collection (ATCC), USA) was grown in monolayer in McCoy's 5A medium supplemented with 10% fetal bovine serum, 2×10^{-3} M L-glutamine and the antibiotics penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were grown in the dark at 37 °C in a 5% CO_2 atmosphere. On regular testing, cell cultures were found to be free from mycoplasma.

2.2. Drugs

Both aclarubicin and 9-aminoacridine were obtained from Sigma (USA), while sodium azide was purchased from Merck (Germany). Aclarubicin was prepared in ethanol and the other drugs were dissolved in distilled water and directly added to the culture