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Role of chromatin structure modulation by the histone deacetylase inhibitor trichostatin A on the radio-sensitivity of ataxia telangiectasia

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ABSTRACT

At present, a lot is known about biochemical aspects of double strand breaks (DBS) repair but how chromatin structure affects this process and the sensitivity of DNA to DSB induction is still an unresolved question. Ataxia telangiectasia (A-T) patients are characterised by very high sensitivity to DSB-inducing agents such as ionising radiation. This radiosensitivity is revealed with an enhancement of chromosomal instability as a consequence of defective DNA repair for a small fraction of breaks located in the heterochromatin, where they are less accessible. Besides, recently it has been reported that Ataxia Telangiectasia Mutated (ATM) mediated signalling modifies chromatin structure. In order to study the impact of chromatin compaction on the chromosomal instability of A-T cells, the response to trichostatin-A, an histone deacetylase inhibitor, in normal and A-T lymphoblastoid cell lines was investigated testing its effect on chromosomal aberrations, cell cycle progression, DNA damage and repair after exposure to X-rays. The results suggest that the response to both trichostatin-A pre- and continuous treatments is independent of the presence of either functional or mutated ATM protein, as the reduction of chromosomal damage was found also in the wild-type cell line. The presence of trichostatin-A before exposure to X-rays could give rise to prompt DNA repair functioning on chromatin structure already in an open conformation. Differently, trichostatin-A post-treatment causing hyperacetylation of histone tails and reducing the heterochromatic DNA content might diminish the requirement for ATM and favour DSBs repair reducing chromosomal damage only in A-T cells. This fact could suggest that trichostatin-A post-treatment is favouring the slow component of DSB repair pathway, the one impaired in absence of a functionally ATM protein. Data obtained suggest a fundamental role of chromatin compaction on chromosomal instability in A-T cells.

1. Introduction

DNA damage is one of the most critical threats to cell survival. To activate multiple signalling pathways at once, sophisticated sensing and transduction systems have evolved to convey the DNA damage signal simultaneously to multiple effectors. The ATM (Ataxia Telangiectasia Mutated) protein plays a central role in such a system, the so called DNA Damage Response (DDR) [1]. ATM is a protein kinase identified as a product of the gene that is lost or inactivated in the disorder ataxia telangiectasia (A-T) [2]. A-T is a human autosomal recessive syndrome characterized by acute cancer predisposition and very high sensitivity to DNA double strand break (DSB)-inducing agents such as ionising radiation (IR), associated with an enhancement of chromosomal instability in cells derived from A-T patients. The clinical and cellular phenotype of the A-T syndrome demonstrates the importance of an appropriate response to DSB induction [3]. ATM signalling is activated by DSB formation and promotes cell cycle checkpoint arrest, apoptosis and affects DSB repair too. DNA non-homologous end-joining (NHEJ) represents the major DSB repair process in mammalian cells that functions throughout the cell cycle [4,5]. Even though A-T cell lines rejoin the majority of DSBs with normal kinetics, demonstrating the most NHEJ occurs independently of ATM signalling [6,7], the rejoining of about 15% of IR-induced DSB require ATM and additional proteins, such as γ-H2AX and 53BP1, that function in the ATM signal transduction cascade [7]. Furthermore, recent studies [8] have demonstrated that DSB persisting in the presence of an ATM inhibitor localise to the heterochromatin suggesting that ATM is required for repairing DSB arising within or close to

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heterochromatic DNA regions. In this context, earlier work on the occurrence of chromosomal aberrations on mitotic chromosomes indicated the induction of more breakpoints in heterochromatin [9] but with methodological progress the opinion shifted rather to a higher occurrence of them in euchromatin [10]. This result was confirmed by directly quantifying DSB using pulsed field gel electrophoresis (PFGE) [11–14]. More recently, this question was readdressed taking advantage of newly available multicolour-fluorescent in situ hybridisation (FISH) observing no difference between the large heterochromatic and euchromatic regions in the frequency of chromosome breakpoints [15]. Besides, Martínez-López and co-workers found a correspondence in the frequency of chromosomal aberrations between the euchromatic short arm and heterochromatic long arm of the X chromosome after etoposide treatment [16].

Even though various studies have suggested that chromatin architecture strongly influences, both, DNA damage induction and its repair, as well as ATM mediated signalling modifies chromatin structure [17,18], at present, there are not conclusive data on radiosensitivity of structurally and functionally different chromatin domains.

In order to study the impact of chromatin compaction on chromosomal instability characteristic of A-T cells, the response to trichostatin A (TSA), an histone deacetylase inhibitor [19], in normal and A-T lymphoblastoid cell lines carrying different mutations in the ATM gene was investigated. TSA, originally isolated as a fungistatic antibiotic from *Streptomyces platensis*, was the first specific natural inhibitor described. Histone deacetylases play a key role in homeostasis of protein acetylation in histones and other proteins thus regulating fundamental cellular activities such as transcription and chromatin structure remodelling [20,21]. TSA can interact with the catalytic site of histone deacetylase resulting in inactivation of this catalytic site and preventing the binding to its substrate [22,23]. Upon TSA treatment a globally increased histone acetylation causing a reversible decondensation of dense chromatin regions leading to a more homogeneous chromatin distribution has been reported [24].

The consequence of TSA chromatin modulation was investigated in normal and A-T lymphoblastoid cell lines testing its effect on chromosomal aberrations (CA) induction, cell cycle progression, DNA damage and repair after exposure to X-rays. In particular, TSA was added to all lymphoblastoid cell lines using different experimental designs: a pre-treatment before X-irradiation in order to determine whether increased histone acetylation could affect the induction and processing of X-rays induced DNA damage; a post-treatment after X-irradiation in order to evaluate whether increased histone acetylation could affect repair of X-rays induced DNA damage; a continuous treatment before and after X-irradiation in order to establish whether pre- and post-treatment could have a synergistic effect. Taken data all together indicate that the reduced radiation-induced clastogenicity found in A-T cells in the presence of TSA could reflect the rejoining of DSB localised in heterochromatic DNA regions favoured by histone hyperacetylation. This suggests a fundamental role of chromatin compaction on chromosomal instability in A-T cells.

2. Materials and methods

2.1. Cell lines and cultures

Lymphoblastoid cell lines (LCLs) AT21RM, AT28RM and AT97RM were established from ataxia telangiectasia patients after immortalization with Epstein Barr Virus (kindly provided by. Prof. L. Chessa, University "La Sapienza" Rome, Italy). At molecular level, AT97RM and AT21RM are homozygous for the deletion CCTC at the codon 717 and for the deletion of 90 nucleotides at the codon 2377 of the *ATM* gene, respectively. AT28RM is a compound heterozygote for a deletion of a CT at the codon 8283 and a truncating substitution C>T at the codon 7792 of the *ATM* gene [25]. FLEBV, presenting a wild type ATM genotype, was used as control lymphoblastoid cell line (also provided by Prof. L. Chessa). Cells were maintained in RPMI 1640 medium (Gibco) supplemented with 20% inactivated foetal bovine serum (FBS) (Gibco), 2% Hepes, 1% Glutamine (Invitrogen) and 1% streptomycin and penicillin (Invitrogen) at 37 °C in a humified atmosphere of 95% air and 5% CO_2. Cell cultures were freshly defrosted before experiments.

2.2. Chemicals

5-Bromo-2-deoxyuridine (BrdUrd), colcemid solution (10 μg/ml) and trichostatin A (TSA) were purchased from Sigma-Aldrich. BrdUrd was dissolved in distilled water at a concentration of 300 μg/ml and used at a final concentration of 6 μg/ml. Colcemid solution was used at a final concentration of 0.2 μg/ml 3 h before cell harvesting. TSA was dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 μM, kept at −20 °C, and used at a final concentration of 50 nM. DMSO final concentration never exceeded 1% in the culture medium.

2.3. Experimental design for cell treatment

Cells were seeded at 8×10^5 cells/ml in RPMI 1640 complete medium 24 h before exposure to X-rays. Cells were X-irradiated at 37 °C with a 250 kV and 6 mA with a Gilardoni MGL 200/8 D X-ray apparatus, at a dose-rate of 60 cGy/min in complete medium, either in presence or absence of 50 nM TSA. Wild-type LCL and A-T LCLs were irradiated with 3 Gy and 1 Gy, respectively to study chromosomal aberration induction while for DNA repair kinetic analysis both wild-type and A-T LCLs were exposed to 3 Gy. TSA was added to all cultures using three different protocols namely: (a) "pre-treatment", where TSA was added 3 h before X-irradiation and removed by washing the cell suspension with phosphate buffered saline (PBS) immediately before X-rays exposure and referred as "TSA-pre"; (b) "post-treatment", where TSA was added immediately after X-irradiation and removed by washing the cell suspension with PBS 3 h after X-irradiation and referred as "TSA-post"; (c) "continuous treatment," where TSA was added 3 h before X-irradiation and then removed by washing the cell suspension with PBS 3 h after irradiation and referred as "TSA-cont". Both TSA dose (50 nM) and treatment time (3 h) have been selected on the basis of existing literature data [26]. To ensure that either pre-treatment, post-treatment or continuous treatment with TSA did not interfere with normal cell cycle progression, a flow cytometric analysis was performed, according to the experimental design (Fig. 1). For chromosomal damage analysis, immediately after X-irradiation, 6 μg/ml BrdUrd was added to the cell suspensions to distinguish first (M_1), second (M_2) mitosis after irradiation. Wild-type LCL and A-T LCLs were sampled at 30 h after X-ray treatment and chromosomal aberrations were analysed in M_1 mitosis after X-ray exposure. This sampling time has been selected as in preliminary experiments the chromosome-type of aberrations (dicentrics) were the predominant type of aberrations, suggesting that the cells were exposed in the G1 phase of the cell cycle. For DNA repair kinetic analysis, the alkaline version of the comet assay, a rapid and sensitive procedure to quantify DNA lesions in mammalian cells [27], was employed to measure the frequency of SSBs and alkali-labile sites in order to study the kinetic of disappearance of DNA breaks as consequences of DNA repair or mis-repair. Wild-type LCL and A-T LCLs were processed at different recovery times (immediately after irradiation, 15, 30, 60 min and 3 h). Consequently the