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Review

Telomeres: Hallmarks of radiosensitivity

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Abstract

Telomeres are the very ends of the chromosomes. They can be seen as natural double-strand breaks (DSB), specialized structures which prevent DSB repair and activation of DNA damage checkpoints. In somatic cells, attrition of telomeres occurs after each cell division until replicative senescence. In the absence of telomerase, telomeres shorten due to incomplete replication of the lagging strand at the very end of chromosome termini. Moreover, oxidative stress and accumulating reactive oxygen species (ROS) lead to an increased telomere shortening due to a less efficient repair of SSB in telomeres. The specialized structures at telomeres include proteins involved in both telomere maintenance and DNA repair. However when a telomere is damaged and has to be repaired, those proteins might fail to perform an accurate repair of the damage. This is the starting point of this article in which we first summarize the well-established relationships between DNA repair processes and maintenance of functional telomeres. We then examine how damaged telomeres would be processed, and show that irradiation alters telomere maintenance leading to possibly dramatic consequences. Our point is to suggest that those consequences are not restricted to the short term effects such as increased radiation-induced cell death. On the contrary, we postulate that the major impact of the loss of telomere integrity might occur in the long term, during multistep carcinogenesis. Its major role would be to act as an amplificator event unmasking in one single step recessive radiation-induced mutations among thousands of genes and providing cellular proliferative advantage. Moreover, the chromosomal instability generated by damaged telomeres will favour each step of the transformation from normal to fully transformed cells.

Keywords: Telomere; DNA repair; Chromosome instability; Radiosensitivity; Radiation induced carcinogenesis

1. Introduction

Telomeres are specialized structures protecting the ends of chromosomes from DSB repair and from activation of DNA damage checkpoints [1]. DNA repair processes interact with telomeres and contribute to telomere maintenance. However, the ways damaged telomeres are processed remain poorly understood. This paper intends to give deep insights into the paradoxical features underlying telomere maintenance and repair of telomeric damage. We will examine the short term and long term consequences of damaged telomeres which are cellular radiosensitive and the promotion of the transmission of radiation induced damage, key steps in radiation-induced tumour progression.

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2. Telomeres and broken ends: role of "stability keepers"

The telomere is composed of TTAGGG repeat tracts associated with specific proteins. Association of telomeric sequences and specific proteins form a high order structure. Telomeric DNA is folded back in d-loop/t-loop structures: telomere's G-rich overhang generated by incomplete replication at the chromosome ends, invades the double-stranded region to form a T-loop. The main function of telomeres is to protect the chromosome ends and to prevent activation of DNA damage response. Defined as the caps of linear chromosomes, they serve to distinguish normal ends from DSBs. Many proteins, involved in DNA repair and checkpoints, are also required for telomere maintenance. Therefore we could wonder how cells are able to discriminate normal from abnormal telomeres. Through the interaction between telomere maintenance and DNA repair, cells develop a sophisticated strategy to detect

eroded or dysfunctional telomeres. Damaged telomere and proper repair failure might result in telomere damage as shown in Fig. 1. It has been reported recently that telomere attrition or dysfunction results in the formation of the hallmark of DNA damage response [2]. Hence, uncapping or senescence elicits the formation of foci including several DNA repair proteins such as 53BP1, H2AX, ATM, Mre11/rad50/NBS1 (MRN) complex, Chk1/2. Correlation between accelerated shortening and hypersensitivity to IR in DSB repair deficiency syndromes argue in favour of a link between telomere maintenance and DNA repair [3].

2.1. Role of NHEJ in telomere maintenance

NHEJ is one of most important pathway in the recognition and processing of DSBs in several organisms. In mammalian cells, NHEJ ensure alignment of DNA ends and ligation by end-joining involving several proteins. NHEJ does not necessarily require sequence homology to join both broken ends. After DSB formation, the complex Ku/DNA-PKcs (DNA-dependent protein kinase catalytic subunit) is involved in initial recognition. Ku binds to DNA ends and recruits DNA-PKcs which can phosphorylate several targets [4]. This is followed by the removal of several base pairs and end-to-end ligation performed by DNA Ligase IV, XRCC4 and XLF [5–7]. Several kinds of IR-induced damage form complex DSBs which would be processed before ligation.

Studies in mammalian cells revealed the role of NHEJ in the protection of chromosome ends. Cells deficient in Ku and DNA-PKcs exhibited premature senescence and high proportions of chromosomal aberrations [8,9]. Absence of Ku86 and DNA-PKcs in mouse cells resulted in an increase of telomere end-to-end fusions with telomeric sequences at the fusion point [10–13]. Both proteins are required for telomere capping in mouse cells. It has been reported that Ku86 acts in telomere homeostasis. Indeed transient inhibition of Ku86 by siRNA caused telomere shortening and telomere dysfunction leading at least to apoptosis [14]. In addition, the suppression of a single allele of Ku86 in somatic cells induced severe telomere shortening associated with telomere fusions [15]. In parallel, deletion of Ku70 leads to an increase of Telomere Sister chromatid Exchange (TSCE) (hallmark of HR events at telomeres) indicating that Ku70 prevents inappropriate recombination at telomeres [16]. Nevertheless, its contribution to telomere length regulation is less clear. Despite the absence of Ku86 affected telomere capping in mouse, data relative to telomere length are conflicting. Abolition of Ku86 displayed a telomere shortening in mouse embryonary fibroblast (MEF) [13] whereas other reports showed a slight elongation of telomeres in the same model [8]. Taken together these data confirm that Ku86 is an essential partner in telomere capping and prevents aberrant recombination events in order to preserve telomere integrity.

Similarly, DNA-PKcs deficiency resulted in telomere uncapping in MEFs. In addition, natural mutations of DNA-PKcs have been observed in severe combined immunodeficiency defects syndrome (SCID) in human and mouse cells. This mutation did not completely abolish DNA-PKcs activity. Cells derived from SCID mouse exhibited abnormal elongation of telomeres and telomere fusions [12,17,18]. Restoration of DNA-PKcs activity restores normal telomere length in SCID [19]. In contrast complete suppression of DNA-PKcs activity by knockout did not change the telomere size [12]. Hence the role of DNA-PKcs in telomere length cannot be clarified yet. However its participation in telomere capping has been reported several times. DNA-PKcs −/− cells showed a significant increase of telomere fusions [20]. In parallel pharmacological inhibition of DNA-PKcs enhanced telomere associations in human cells and confirmed the importance of DNA-PKcs in telomere homeostasis [20]. Moreover, chromosome orientation FISH (CoFISH) studies revealed that in the absence of DNA-PKcs, fusions occurred between both leading strands after replication suggesting that DNA-PKcs was involved in post replicative telomere capping [20].

Other components of NHEJ have been shown to participate in telomere maintenance. DNA Ligase IV is often described as a potential actor at telomeres. DNA Ligase IV, involved in the ligation of DNA broken ends, is essential in mammalian cells. Its suppression is lethal in embryogenesis but invalidation of P53 in mouse cells rescue lethality of DNA Ligase IV −/− [21]. In contrast, studies on two cell lines from patients affected by DNA Ligase IV mutation revealed an accelerated shortening comparable to AT (ataxia-telangiectasia) cells [3]. A mouse strain with a hypomorphic mutation shows that diminished DNA double-strand break repair leads to adult stem cell exhaustion over time [22]. However the reduced

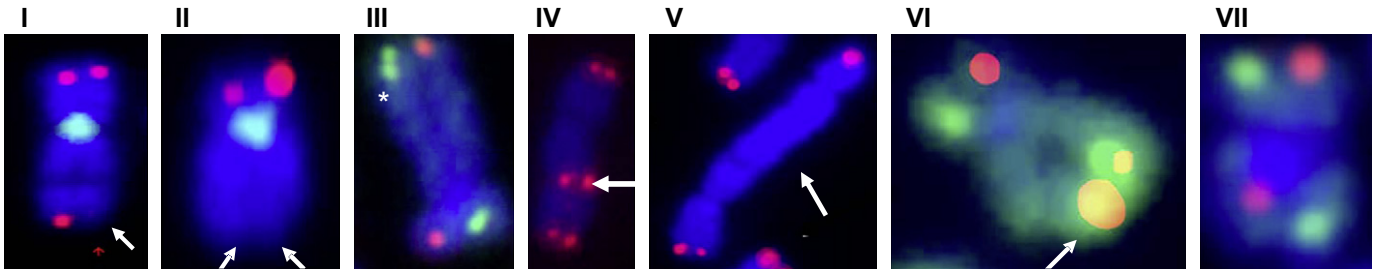

Fig. 1. Telomere dysfunctions in human cells. Metaphase spread with telomeric DNA detected by FISH (red); DNA stained with DAPI (blue). Examples of the chromosomal aberrations: (I) Loss of single telomere (STL). (II) Loss of both telomeres. (III) Telomeres split/duplication. (IV, V) Dicentrics chromosomes with (IV) or without (V) TTAGGGq sequences at fusions junction. (VI, VII) CoFISH staining with C-rich probe (red) and G-rich (green) probe with (VI) or without telomere-SCE (T-SCE) (VII).