CMB PhD School Seminars 2022-2023

3rd October 2023, 2:30 p.m.

Aula Magna anatomia Olivo, via Irnerio 48, ground floor

Online meeting on Teams

https://teams.microsoft.com/l/meetup-join/19%3ameeting_MGI2OTJhNzYtYzM4Ny00NTVmLWFlZmUtMWQ5YWQxM2UyNWU4%40thread.v2/0?context=%7b%22Tid%22%3a%22e99647dc-1b08-454a-bf8c-699181b389ab%22%2c%22Oid%22%3a%22c15c26f4-3c3b-4517-8cbc-bf8243e6b95b%22%7d

Impaired formation of a stable RPA/RnaseH1 complex in senescent cells leads to uncontrolled processing of R-loops and unsuccessful DNA repair

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The 40-minutes scientific talk by Prof. Di Giorgio will be followed by a 30-minutes "Meet the speaker" Q&A session with the PhD students

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Abstract

The maintenance of genome stability ensures cellular fitness. Cells are constantly exposed to DNA-damaging agents and DNA integrity is maintained by successful activation of the DNA damage response (DDR). Senescent cells accumulate unrepaired DNA due to a defective DDR. Noncanonical chromatin structures (NCSs) enable the maintenance of genome integrity by controlling genome replication, transcription, and DNA repair. Among NCSs, R-loops are three-stranded DNA-RNA hybrids that have been reported to recruit repair factors and maintain successful DNA repair when formed *in trans* and to increase genome instability when formed co-transcriptionally *in cis*. However, the reason for this apparent paradox has not been fully understood yet.

We have recently found that cells undergoing oncogene-induced senescence (OIS) and replicative senescence (RS) accumulate more R-loops than proliferating fibroblasts. The accumulation of these R-loops during senescence is due to the subversion of the epigenetic balance that controls the turnover from acetylation to ubiquitination of the core histone H2B at lysine 120. In pre-senescent cells subjected to replication stress, the persistence of R-loops at double-strand breaks (DSBs) prevents successful loading of BRCA1 and RAD51 and execution of homologous recombination repair. By crossing DRIP-seq and DRIVE-seq data, we found that impaired loading of DDR proteins at DSBs correlates with unsuccessful recruitment of RNAseH1 by the RPA complex to R-loops. In proliferating cells, phosphorylation of RPA32 at S4/8 controls the release of RNAseH1 from the lesion, but in cells subjected to replication stress, hyperphosphorylation of RPA32 leads to premature release of RNAseH1, accumulation of R-loops, and senescence entrance. By using the LacO/LacR tethering system, we found that the forced recruitment of both catalytically active and inactive RnaseH1 to DSBs delays DNA repair, suggesting that both premature and delayed removal of R-loops affects DDR.

Finally, our research identified phosphorylation of RPA32 as the signal that controls the release of RNAseH1 from DSBs. Hyperphosphorylation of the RPA complex observed in pre-senescent cells alters this mechanism, slows down end-resection and contributes to further accumulation of irreparable DNA damage and onset of senescence

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