Nucleosome disruption by 5-bromodeoxyuridine leads to senescence

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Abbreviations

SASP, senescence-associated secretory phenotype; BrdU, 5-Bromodeoxyuridine; SA-β-gal, senescence-associated-β-galactosidase; ChIP, chromatin immunoprecipitation.

ABSTRACT

5-Bromodeoxyuridine (BrdU) exposure leads to senescence, but the mechanistic details remain elusive. In this issue, En *et al*. [1] unveil a role of the HBR domain in histone H2B as a potential mediator of the effects of BrdU both in yeast and in human cells.

Introduction

Senescence is a cellular stress response to halt the expansion of damaged cells. Described for the first time more than 60 years ago, senescence was initially dismissed as a cell culture artifact [2]. However, years of intense research have proved a myriad of physiological and pathophysiological roles for senescence, not just in animal models but also in human disease. Well characterised examples include developmental senescence [3, 4], senescence as a tumour suppressor mechanism [5] and, more recently, senescence as a driver of age-related diseases [6, 7].

Cellular senescence is a complex and dynamic state leading to phenotypic changes such as irreversible cell growth arrest, the acquisition of a senescence-associated secretory phenotype (SASP), and an increase in lysosomal mass and activity [8, 9]. Senescent cells appear in response to a multitude of stressors including telomere erosion, oncogenic activation, DNA damage, mitochondrial dysfunction, or epigenetic modifications [10]. Importantly, therapeutic strategies targeting senescent cells are now moving from preclinical studies to clinical trials, highlighting the need to expand our understanding of the molecular basis for senescence to fuel the benefits of these therapies while keeping their side effects to a minimum.

5-Bromodeoxyuridine (BrdU) is an analogue of the nucleoside thymidine used as a proliferation marker. The assay is based on the incorporation of BrdU in the DNA of dividing cells during the S-phase of the cell cycle. Interestingly, BrdU exposure also induces senescence in mammalian cells [11], but the mechanistic details of this association are poorly understood.

Deciphering how BrdU leads to senescence

The work presented by En *et al*. in this issue of *The FEBS Journal* aims to shed light on how BrdU destabilizes nucleosome positioning leading to senescence.

Nucleosomes consist of a section of DNA wrapped around a histone octamer, composed of two copies of each of the histone proteins: H2A, H2B, H3, and H4. Previous studies using a specific strain of *S. cerevisiae* showed that exposure to BrdU restrains its growth, potentially by triggering histone modifications [12, 13]. Here, the authors specifically explore if histone N-terminal tails account for BrdU sensitivity in yeast.

Yeast cells expressing a mutant version of H2B lacking its N-terminal tail ($H2B^{\Delta N}$) cease proliferation when exposed to BrdU with a higher sensitivity than those expressing an empty vector. Interestingly, the H2B N-terminal tail includes a highly conserved region, the HBR domain, with a role in gene repression. When En *et al*. expressed in yeast a mutant version of H2B lacking its N-terminal tail but preserving the HBR domain (HBR-$H2B^{\Delta N}$), BrdU sensitivity reverts to normal levels. Therefore, these results suggest that the lack of the HBR domain in the H2B N-terminal tail explains the enhanced sensitivity to BrdU in yeast. To further confirm this finding, the authors looked at the transcriptional profile of yeast cells in response to BrdU exposure *versus* yeast cells with a deletion of the HBR domain. Results from two independent cDNA microarray assays show that around 50% of the genes upregulated in response to BrdU are also upregulated in response to the deletion of the HBR domain in histone H2B. Moreover, both deletion of the HBR domain and BrdU exposure destabilize nucleosome positioning and upregulate gene expression in yeast, pointing to a shared mechanism of action.

Next, En *et al*. tested whether the HBR domain in histone H2B N-terminal tail also mediates BrdU sensitivity in human cells. Only when expressing a mutant version of H2B lacking the HBR domain, HeLa cells stop dividing after exposure to a low dose of BrdU. Most of all, and beyond the cell growth arrest, these human cells display other

features of senescence like an enlarged and flattened cell morphology, an increase in the percentage of senescence-associated-β-galactosidase (SA-β-gal) positive cells, and the expression of certain SASP components (Fig. 1).

In yeast, the lack of the HBR domain destabilizes the nucleosomes rendering them more susceptible to nuclease digestion. Similarly, in HeLa cells the lack of the HBR domain combined with exposure to a low dose of BrdU increases the sensitivity to nuclease digestion. Therefore, the loss of the HBR domain may also affect the nucleosome arrangement in human cells. Furthermore, yeast cells missing the HBR domain upregulate the expression of certain genes with reduced histone occupancy. Hence, En *et al*. assessed the effects of a high dose of BrdU on histone H2B occupancy in HeLa cells using chromatin immunoprecipitation (ChIP) analysis. Among the upregulated genes after exposure to BrdU, the authors found that at least *IL-8*, *SERPINE1*, and *SERPINE2* show decreased H2B occupancy at their promoter site. Altogether, this reinforces the idea that the consequences of BrdU exposure on human cells might be a result of its effects on nucleosome organization. Even a low dose of BrdU combined with the lack of the HBR domain was enough to upregulate the expression of *IL-8* and *SERPINE1* while decreasing H2B occupancy. A common feature of these two genes is their high A/T content in their promoter region. Of note, A/T sites are the targets of BrdU as intercalating agent. Consequently, those genes with a higher A/T content are more sensitive to the effects of BrdU.

Overall, the findings presented in this study suggest that BrdU not only acts as a DNA intercalating agent in A/T-rich sites, but also indirectly affects gene expression by targeting the HBR domain in histone H2B, disrupting nucleosome positioning and leading to senescence. Future research should explore whether BrdU exposure also induces senescence in pre-clinical models, for instance, as part of a “one-two punch”

strategy to treat cancer, i.e., pro-senescence therapy followed by a senolytic compound [14]. Nevertheless, and due to the high toxicity of BrdU, subsequent studies should search for alternative strategies to induce senescence, for example, screening for small molecule antagonists of the HBR domain.

Conclusion

The realisation that senescent cell removal reverts age-related phenotypes alongside the role of senescence in tumour suppression highlight the relevance of this once overlooked cellular state. To clear the way for successful clinical translation, efforts towards a better understanding of the molecular intricacies of senescence are needed. Besides the well-known DNA damaging agents as triggers of senescence, the potential to induce senescence of changes in nucleosome positioning and other epigenetic modifications warrants further investigation.

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CONFLICT OF INTEREST

AG is a named inventor in an MRC patent related to senolytic therapies.

AUTHOR CONTRIBUTION

AG wrote the manuscript.

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FIGURE LEGEND

Figure 1. Exposure to a high dose of BrdU (50 μM) induces senescence in HeLa cells. The study by En *et al*. suggests this is mediated, at least in part, by loosening the interaction of the HBR domain in histone H2B with the DNA. Accordingly, even a low dose of BrdU (5, 15 μM) triggers senescence if combined with a deletion of the HBR domain in histone H2B. This Figure was created with *BioRender.com*.

BrdU (high)

or

BrdU (low) + HBR deletion