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The oxidative DNA damage response: A review of research undertaken with Tsinghua and Xiangya students at the University of Pittsburgh

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Endogenous stress and exogenous toxicants (chemicals and UV light) alter genetic information either directly or indirectly through the production of reactive oxygen species (ROS), thereby driving genomic instability in cells and promoting tumorigenesis. All living cells try to faithfully preserve and transmit their genomic information from one generation to the next using DNA repair mechanisms to repair oxidative DNA damage to prevent cancer or premature aging. Oxidative DNA damage comprises a mixture of DNA lesions including base damage, DNA single strand breaks (SSBs), and DNA double strand breaks (DSBs). This review summarizes some of the studies on DNA damage response at a defined genome locus that are performed by students from the Tsinghua University School of Medicine and the School of Medicine of Central South University (Xiangya Hospital) at the University of Pittsburgh School of Medicine. A summary of their work highlights the continuous contribution of the students to a particular research program and exemplifies the achievements of this China-U.S. collaborative training program.

DNA damage, chromatin, telomere, DNA repair

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As described in a companion report ("Sino-U.S. Partnerships in Research, Education, and Patient Care: The Experience of the University of Pittsburgh and UPMC"), students from the Tsinghua University School of Medicine and the School of Medicine of Central South University (Xiangya Hospital) have spent two years of their medical school curriculum participating in research at the University of Pittsburgh School of Medicine, with the first class at Tsinghua arriving in 2012 and that of Xiangya in 2014. Among the authors of this review, with years in parentheses indicating time spent at the University of Pittsburgh, LS (2012-2014), YG (2013-2015), LY (2014-2016), YT (2016-present), and HC (2016-present) are Tsinghua students (years at the University of Pittsburgh). RT (2013-2016) is a Xiangya student who undertook her research in Pittsburgh as a collaborative project. All of these co-authors spent their research time in our laboratory (LL, ASL) at the UPMC Hillman Cancer Center and their projects were focused on various aspects of genome and telomere stability in the wake of oxidative DNA damage. A summary

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of their work, which is described in chronological order, exemplifies the achievements of this China-U.S. collaborative training program and highlights the continuous contribution of the students to a particular research program, again as one example of the productivity of these partnerships.

Environmental toxicants (chemicals and UV light) alter genetic information either directly or indirectly through the production of reactive oxygen species (ROS), thereby driving genomic instability in cells and promoting tumorigenesis. All living cells try to faithfully preserve and transmit their genomic information from one generation to the next using DNA repair mechanisms to repair ROS-induced DNA damage; an abnormal elevation in ROS produces oxidative stress, which also contributes to premature aging (Hegde et al., 2012). ROS-induced damage comprises a mixture of DNA lesions including base damage, DNA single strand breaks (SSBs), and DNA double strand breaks (DSBs) (Figure 1A). To study oxidative DNA damage response at a single genome locus in different genomic locations with various molecular processes, we constructed the DART (Damage at Active RNA Transcription Sites) system or DART at telomeres (Figure 1B). As illustrated in Figure 1C, a tandem tetracycline repressor (tetR) array cassette was integrated at a defined genomic locus in U2OS TRE cells. Radiation and chemically-induced oxidative stress give rise to oxidative DNA damage throughout the genome, preventing analysis of damage repair specifically at transcriptionally active sites. We took advantage of the KillerRed (KR) protein as an inducible source of localized damage. KR is a modified red fluorescent protein chromophore, which generates superoxide when exposed to visible light. KR mimics radiation-induced oxidative DNA damage by generating oxygen radicals. The DART system (Figure 1C), combined with genome site-specific KR positioning, is a unique and precise approach that allows oxidative damage to be introduced at a specific genomic site. We activated KR in cultured cells by exposing them to a 15 W white fluorescent bulb on an exposure stage (Uvland, CA). The precise local damage delivery and capability for dose modulation in this approach were ideal to carry out the experiments described below. Thus, the DART systems avoid the confounding effects of global and random DNA damage associated with cell exposure to chemical compounds and radiation (Lan et al., 2014; Wei et al., 2015).

LS and RT further developed the DART system to induce oxidative damage at individual telomeres (Figure 2) (Sun et al., 2015). Cellular DNA is organized into chromosomes and capped by a unique nucleoprotein structure, the telomere. Both oxidative stress and telomere shortening/dysfunction cause aging-related degenerative pathologies and increase cancer risk. However, a direct connection between oxidative damage to telomeric DNA, comprising <1% of the genome, and telomere dysfunction had not been established. By

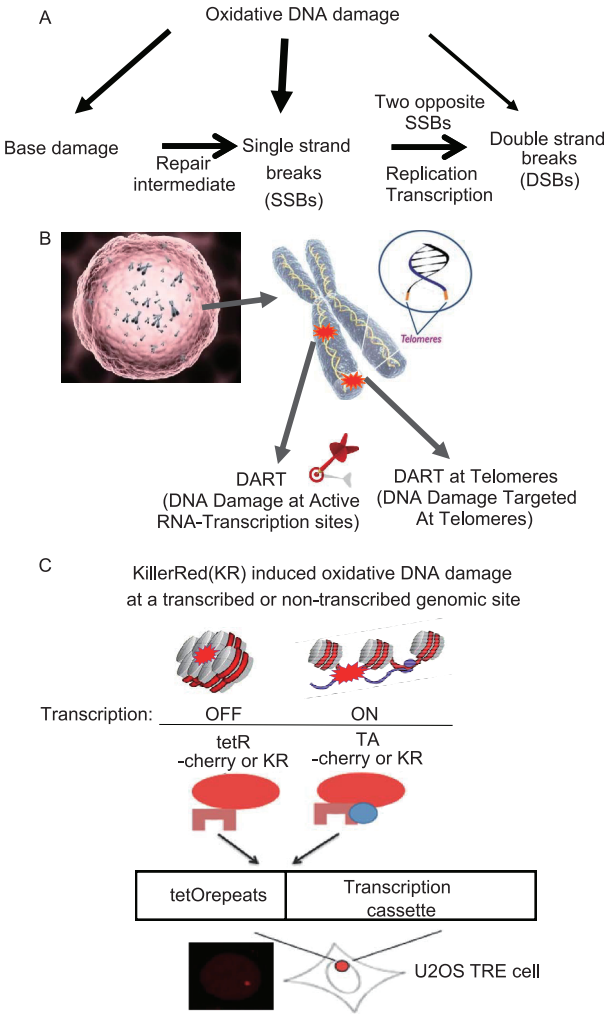

Figure 1 Scheme of type of DNA damage and damage inducing system. A, Types of oxidative DNA damage. B, Illustration of the DART systems. C, Scheme of the KillerRed/DART system for inducing genome locus-specific damage.

fusing the KillerRed chromophore gene to the telomere repeat binding factor 1 gene (*TRF1*), a novel approach was established to generate localized damage to telomere DNA and to monitor the real time damage response at the single telomere level. LS and RT found that DNA damage at long telomeres in U2OS cells is not repaired efficiently compared to DNA damage in non-telomeric regions of the same length in heterochromatin. Telomeric DNA damage shortens the average length of telomeres and leads to cell senescence in HeLa cells and cell death in HeLa, U2OS and IMR90 cells, when DNA damage at non-telomeric regions is undetectable. Telomere-specific damage induces chromosomal aberrations, including chromatid telomere loss and telomere associations, distinct from the damage induced by ionizing irradiation. Taken together, these results demonstrated that oxidative damage induces telomere dysfunction and underlined the importance of maintaining telomere integrity upon oxidative damage. Figure 2 demonstrates the model from this work, in

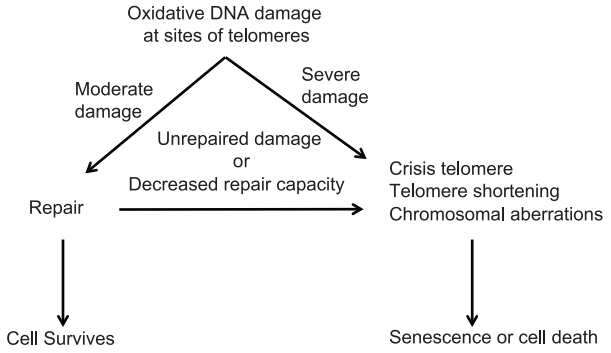

Figure 2 A model depicting oxidative DNA damage at telomeres leading to cell death. Telomere-specific oxidative damage disrupts telomere integrity and accelerates telomere shortening.

which the evidence that telomere-specific DNA damage could disrupt telomere integrity and lead to cell death was shown for the first time.

Following this work, RT discovered a mechanism underlying efficient protection of telomeres from damage that depends on Nek7-dependent stabilization of TRF1 (Tan et al., 2017). TRF1 is essential to the maintenance of telomere chromatin structure and integrity. However, how telomere integrity is maintained, especially in response to damage, had been poorly understood. In this work, RT identified Nek7, a member of the Never in Mitosis Gene A (NIMA) kinase family, as a regulator of telomere integrity. Nek7 is recruited to telomeres and stabilizes TRF1 at telomeres after damage in an ATM activation-dependent manner. Nek7 deficiency leads to telomere aberrations, long-lasting γH2AX and 53BP1 foci, and augmented cell death upon oxidative telomeric DNA damage. Mechanistically, Nek7 interacts with and phosphorylates TRF1 on Ser114, which prevents TRF1 from binding to Fbx4, an Skp1-Cul1-F box E3 ligase subunit, thereby alleviating proteasomal degradation of TRF1, leading to a stable association of TRF1 with Tin2 to form a protective shelterin complex.

Meanwhile, LS focused her project on understanding how oxidative DNA damage at telomeres triggers aging in Werner Syndrome (WS), one of the progeroid disorders associated with premature aging (Sun et al., 2017). WS is caused by *WRN* gene mutations. WS cells exhibit shorter telomere length compared to normal cells, but it had not been fully understood how *WRN* deficiency leads directly to telomere dysfunction. With the DART system which induces telomere-specific DNA damage in a real-time fashion and a dose-dependent manner, LS found that the damage response to WRN at telomeres relies on its RQC domain, which is different from the canonical damage response at genomic sites associated with WRN's HRDC domain. In addition to steady state telomere erosion, WRN depleted cells are also sensitive to telomeric damage. Thus, LS's work revealed a novel mechanism as to how WRN protects telomere integrity from damage and telomere erosion.

Oxidative DNA damage triggers telomere erosion and cellular senescence as noted in the studies above. However, how repair is initiated at telomeres remained largely unknown. In studying this process, LY and LS found that unlike PARP1-mediated PARylation at genomic damage sites, PARylation at telomeres is mainly dependent on tankyrase1 (TNKS1) (Yang et al., 2017). TNKS1 was recruited to damaged telomeres via its interaction with TRF1, which subsequently facilitated the PARylation of TRF1 after damage. TNKS inhibition abolished the recruitment of the proteins that repair DNA SSBs, X-ray Repair Cross-Complementing Protein 1 (XRCC1) and polymerase beta, at damaged telomeres, while the PARP1/2 inhibitor only had such an effect at non-telomeric damaged sites. The ANK domain of TNKS1 is essential for the telomeric damage response and TRF1 interaction. Mutation of the tankyrase-binding motif (TBM) on TRF1 (13R/18G to AA) disrupts its interaction with TNKS1, inhibiting recruitment of TNKS1 and repair proteins after damage. Either TNKS1 inhibition or TBM mutated TRF1 expression markedly sensitized cells to telomere oxidative damage as well as XRCC1 inhibition. Together, these data revealed a novel role of TNKS1 in facilitating SSB repair at damaged telomeres through PARylation of TRF1, thereby protecting genome stability and cell viability. Figure 3 is a model of poly ADP-ribosylation mediated by different enzymes at non-telomeric versus telomeric regions. In addition, whether oxidative damage induces telomere movement and how telomere mobility is regulated remains poorly understood. YG has addressed telomere movement in the absence or presence of oxidative damage using the DART at Telomere system (manuscript to be submitted).

To elucidate the upstream regulator of repair of SSBs, YG identified a novel role of structure-specific recognition protein-1 (SSRP1). Repair of SSBs requires overcoming the chromatin barrier for efficient initiation, and this is critical for maintaining genome stability. SSRP1 is the major com-

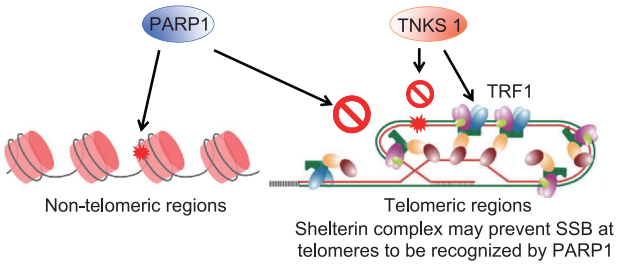

Figure 3 A model of poly ADP-ribosylation mediated by different proteins, at distinct genome loci, recruiting the oxidative damage repair machinery. Oxidative DNA damage at genome sites and telomeres facilitates the formation of PAR via PARP1 and TNKS1, respectively. TNKS1 is recruited to damaged telomeres through its interaction with TRF1. Subsequent PARylation of TRF1 by TNKS1 recruits downstream repair proteins to sites of damage, promoting genome stability and cell survival.

ponent of the facilitate chromatin transcription (FACT) complex, which functions as a histone H2A/H2B chaperone, promoting chromatin disassembly during transcription, replication, and repair processes. However, if and how the two components of FACT (SSRP1 and SPT16) are involved in the repair of DNA SSBs and the promotion of chromatin priming was largely unknown. YG showed that SSRP1 but not SPT16 is critical for cell survival after ionizing radiation and methyl methanesulfonate induced damage. SSRP1 was recruited at SSBs in a PARP activation-dependent manner. SSRP1 was retained at sites of damage after PAR degradation and interacted with XRCC1, the scaffold protein of SSBR. The N-terminus conserved domain of SSRP1 interacted with the N-terminus of XRCC1. Mutation of the ALFFSRI RIR motif on XRCC1 (FF191/192AA) greatly abolished SSRP1's interaction with XRCC1. Most importantly, the function of SSRP1 is essential for chromatin decondensation and promoting histone H2B exchange at sites of DNA strand breaks, which is critical for priming chromatin for efficient SSB repair and cell survival. These results indicate that SSRP1 cooperates with XRCC1 after PARP activation and facilitates SSB repair by promoting chromatin priming through histone exchange, and provide a mechanistic basis for future cancer therapeutic approaches targeting SSRP1 (Gao et al., 2017).

Two of the Tsinghua students (YT and HC) have contributed to several of these projects, and their ongoing projects are also related to the general theme of DNA damage response and genomic stability. Their studies are expected to add significantly to the results summarized in this review, reflecting the productive continuum of the partnership between the University of Pittsburgh School of Medicine and Tsinghua University School of Medicine.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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