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Nucleus versus Nucleolus Damage-Impacts of DNA damage levels on p16^{INK4a} expression after Laser Ablation

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Extended Abstract

Cellular senescence, the state where cells permanently stop dividing, plays a critical role in chronic lung diseases like idiopathic pulmonary fibrosis (IPF), which carries a median survival of only three years.[1] Senescence is triggered by stressors including DNA damage, oxidative stress, and telomere attrition, and is marked by the cyclin-dependent kinase inhibitor p16^{INK4a}, identified in lung fibroblasts, plays an important role in lung regeneration. Senescent cells release the senescence-associated secretory phenotype (SASP), which drives inflammation and can propagate senescence in neighboring cells. Emerging senolytic therapies such as dasatinib and quercetin have shown early promise in IPF patients, but the mechanisms by which localized DNA damage triggers and spreads senescence remain poorly defined.[2] This study aims to observe how different levels of laser-induced damage affect cells and their surroundings, using *p16^{INK4a}* expression and γH2AX signaling as readouts of the senescent response to DNA damage.

To induce localized DNA damage, cultured primary lung fibroblasts were exposed to a 780nm Femtosecond laser at 75 mW and 100mW, measured prior to passing through a 40x 1.2NA objective on Zeiss Axiovert 200M microscope. Lung fibroblasts from a *p16^{INK4a}* reporter mouse, INKBRITE, were used to identify non- expressing fibroblasts that were DNA damaged before inducing damage. 24 hours after injury, cells were fixed and immunolabeled for γH2AX and reporter gene. DNA damage response (DDR) was quantified through the quantification of γH2AX foci and average nuclear intensity using ImageJ. In addition to cut-cells, non-cut surrounding cells with the same imaging field were selected for analysis as internal controls.

Laser-induced DNA damage significantly elevated p16^{INK4a} expression in cut cells after 24 hours in both the nucleolar and nuclear regions. While the nucleus serves as the primary site of the DNA storage and repair, the nucleolus is a dense subdomain involved in ribosome biogenesis and may exhibit distinct stress responses. The targeted damage increased significantly in p16^{INKa} expression after 24 hours, reaching 20,000 – 25,000 AU compared to near-zero in p16^{INK4a}-negative no damage controls ($p<0.0001$). γH2AX mean intensity increased with laser power, from 5,000 – 8,000 AU at 75mW to 10,000 – 13,000 AU at 50mW and 20,000 – 25,000 AU at 100mW, with γH2AX foci count and max intensity following the same trend. Nucleolar-targeted damaged cells, resulted in a lower γH2AX compared to the p16^{INK4a}, suggesting nuclear disruption, resulting in a prominence of senescence. Despite dose-dependent DNA damage escalation, p16^{INK4a} expression did not differ significantly between the 75mW and 100mW groups, suggesting the senescence threshold was already reached at lower power when the nucleolus was damaged. These findings help define laser settings for studying paracrine senescence signaling and may inform therapies targeting senescent cells in the tumor microenvironment.

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