

"Mechanisms of Micronuclear Envelope Rupture, and Impact to Genomic Stability"

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AY 2019-2020 Progress Report

Background & Significance

Astronauts on longer space flights have significantly more chromosomal aberrations in their blood lymphocytes as a result of space radiation exposure, with one common defect being the formation of micronuclei following radiation induced DNA breaks. Micronuclei contain chromosomes excluded from the main nucleus and are often used clinically as a marker of genomic instability, are closely linked with chromosome defects, and are associated with an increased risk of cancer development. However, it is not clear how micronuclei contribute to pathogenesis at the cellular level. Furthermore, micronuclei can form through several mechanisms, and it is not understood whether all micronuclei have similar effects on cellular fitness. Our research will determine if micronuclei formed due to radiation pose similar risks to astronauts as micronuclei formed through other pathways. This will allow NASA to better assess risks to genome preservation and cancer development due to radiation exposure for astronauts on long space missions.

Project Goals

It is believed that micronucleated chromosomes can become damaged and lead to genomic instability by fragmenting during the next mitotic division or by becoming exposed to the cytoplasm after micronuclear envelope rupture. Either of these events are predicted to disrupt genome stability. However, it is not understood if micronuclei forming via various pathways are similarly predisposed to these types of damage. The goals of this project are to determine if differently formed micronuclei pose the same pathogenic risks to an individual, and whether micronuclei formed due to radiation pose similar risks to astronauts as micronuclei formed through other pathways. We have utilized a novel mouse model paired with a physiologically relevant way to genetically induce micronuclei in an *in vitro* human cell culture system to answer these questions.

Summary of Key Findings

Micronuclei forming as a result of chromosome alignment defects exhibit stable nuclear envelopes. I investigated rates of nuclear envelope rupture for micronuclei formed through three distinct cellular insults: 1) inducing improper attachments between chromosomes and mitotic spindle microtubules via the drug nocodazole; 2) disrupting alignment of chromosomes before segregation by removing KIF18A; and 3) creating DNA breakages via exposure to radiation. I found that micronuclei formed due to improper attachments and radiation are more likely to rupture (nocodazole treatment: 59%; 1Gy: 26% rupture), than those that form due to unaligned chromosomes (KIF18A knockout;

15% rupture). Micronuclei in KIF18A knockout (KO) cells also formed close to the main chromatin mass, suggesting the subcellular location of formation affects stability (see figure).

Micronuclei that form near the main chromatin mass exhibit successful chromatin expansion upon exit from mitosis. Chromosomes within micronuclei that form due to unalignment decondensed upon exit from mitosis, as measured by an increase in chromatin area in live cells. This indicates successful expansion and stabilization of the micronuclear envelope. Expansion analyses, together with positioning data (above), suggests that proximity to the ER, an important contributor to the nuclear membrane, is an important positive effector of micronuclear envelope stability.

Tumors from *Kif18a/p53* mutant mice exhibit increased micronuclear frequency and reduced survival. We observed higher levels of micronuclei in the tumors from mice lacking both *kif18a* and *p53* (5.5%) than those lacking *p53* alone (3%, $p < 0.001$). Survival of *Kif18a*^{-/-}/*p53*^{-/-} mice were moderately reduced, compared to *p53*^{-/-} mice ($p = 0.01$). If genomic instability is introduced through fragmentation of micronucleated DNA during the next mitotic cell cycle, we expect to observe greater levels of genomic instability in *Kif18a/p53* mutant mice than in *p53* mutants alone. We are currently conducting sequencing experiments to answer this question.

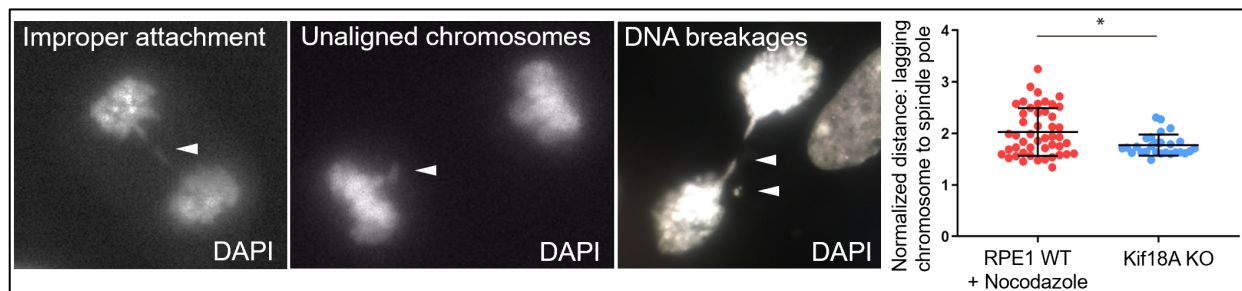


Figure: Different mechanisms for micronucleus generation result in micronuclei forming in different subcellular locations. Left: The frequency of lagging chromosomes (indicated by white arrow) varies significantly between mechanisms of micronucleus formation. Lagging chromosomes, which form micronuclei, were observed at the center of 44% and 35% of dividing cells following nocodazole treatment and irradiation, respectively, compared to only 9% in KIF18A knockdown cells. DAPI is a DNA-specific fluorescent stain, allowing imaging of chromosomes using fluorescence microscopy. Right: Chromosomes positioned greater than 2x beyond the standard deviation of average chromosome distance to spindle pole (calculated for each cell) were identified as “late-lagging chromosomes”; normalized position of late lagging chromosomes is plotted. (Statistics determined via Student’s t-test; * = significance: $p < 0.01$.)