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

Leslie Sepaniac; Department of Molecular Physiology and Biophysics
AY 2018-2019 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.

Summary of Key Findings

There are two non-mutually exclusive models to explain how micronuclei contribute to genomic instability: micronucleated chromosomes 1) fragment during the next mitotic division and/or 2) become exposed to the cytoplasm after micronuclear envelope rupture. I investigated the first hypothesis by focusing on the fate of cells forming micronuclei following three distinct cellular insults: 1) inducing improper attachments between chromosomes and mitotic spindle microtubules via knockdown of an essential mitotic spindle assembly checkpoint protein, Mad2; 2) disrupting alignment of chromosomes before segregation by removing KIF18A, a motor protein; and 3) exposing cells to sub-lethal radiation. Pervious investigations of micronuclei have been performed in cells lacking the tumor suppressor, p53. To determine whether the mechanism of micronucleus

formation, or active tumor suppressor processes impact micronucleus stability, I performed all experiments using cells normally expressing or depleted of p53. Using live cell imaging, I demonstrated that micronucleated cells resulting from unaligned chromosomes undergo p53-dependent cell cycle arrest (division reduced by 67%), suggesting that active processes prevent micronucleated cells from dividing subsequently. These data challenge the idea that a micronucleus alone is enough to induce chromosome fragmentation and instead indicate that an additional "gateway" mutation is necessary (Fonseca et al., *Journal of Cell Biology* doi: 10.1083/jcb.201807228). Further, I investigated the rates of micronuclear envelope rupture for micronuclei formed through different mechanisms. I found that micronuclei forming due to improper attachments and radiation are more likely to rupture (nocodazole treatment: 29% rupture; 1 Gy: 26% rupture), compared to those that form due to unaligned chromosomes, (KIF18A knockout; 15% rupture), indicating the fate of micronuclei differs between mechanisms. The rates of rupture are not significantly affected by p53 knockdown in any condition, but I found a strong correlation between rupture and the subcellular location of micronucleus assembly (see figure).

Next, I will test two non-mutually exclusive pathways predicted to explain this observation. Through a collaboration with Dr. Laura Reinholdt at The Jackson Laboratory, we have created a mouse model for this study by crossing *kif18a* mutant mice with mice lacking the key cell cycle regulator *p53*. These mice produce lymphoma tumors, in keeping with their *p53* null background, and micronuclei, as a result of unalignment (via removal of *kif18a*). If the model that genomic instability is introduced through fragmentation of micronucleated DNA during the next mitotic cell cycle is correct, we expect to see greater levels of genomic instability in these crossed mice (lacking both *kif18a* and *p53*) compared with *p53* mutant, but *kif18a* normal, mice. We are collaborating with the UVM Genomics Core to sequence and analyze whole genome sequences from extracted murine tumor cells, in order to evaluate resulting genomic stability across these different genotypes. Experiments are ongoing.

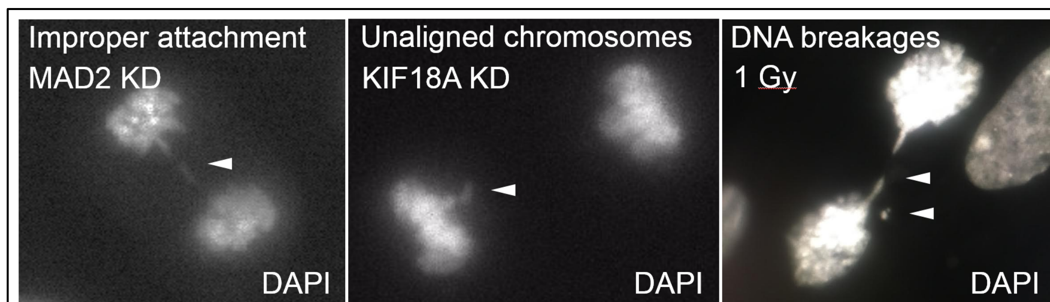

Figure: Different mechanisms for micronucleus generation result in micronuclei forming in different subcellular locations. The frequency of lagging chromosomes (indicated by white arrow) varies significantly between mechanisms of micronucleus formation. Lagging chromosomes, which form micronuclei, were observed at the center of 44% and 35% of dividing cells following MAD2 knockdown 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.