Investigating the Molecular Origins of High-Grade Serous Ovarian Cancer

CancerCare MANITOBA
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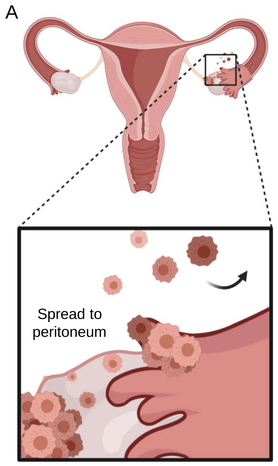
INTRODUCTION

High-grade serous ovarian cancer (HGSOC) is the most common subtype of epithelial ovarian cancer (EOC), accounting for ~70% of all EOC diagnoses. Currently the early etiological events of HGSOC are poorly understood, and as a result there are few early detection methods, making HGSOC the most lethal gynecological cancer. Chromosome instability (CIN) is defined as the increase in the rate at which whole chromosomes, or large chromosomal fragments are gained or lost. In many cancers, CIN is associated with tumor initiation and development, intra/inter-tumoral heterogeneity, multi-drug resistance, and poor patient outcomes. Preliminary data from the McManus laboratory has determined that reduced expression of two SCF complex members, *SKP1* and *RBX1*, induces CIN in fallopian tube (FT) secretory epithelial cells, a cell of origin for HGSOC (Figure 1A). The SCF complex (Figure 1B) is an E3 ubiquitin ligase that functions in maintaining genome stability and is implicated in the DNA damage response pathway, a critical pathway in response to genotoxic stress. In this regard, ovulation induces inflammation and the production of reactive oxygen species, which induces genotoxic stress and DNA double-stranded breaks. If not repaired correctly, DNA double-stranded breaks can cause novel mutations that are transmitted to all daughter cells. Previous genetic studies have shown that ovulation induced genotoxic stress is implicated in the development of epithelial ovarian cancer, however, its impact on CIN and the development of HGSOC is unknown. Accordingly, the current study aims to investigate the molecular origins of HGSOC, by determining the impact of genotoxic stress in the form of ionizing radiation (IR) on surrogate markers of CIN.

Figure 1. Aberrant SCF Complex Function Induces CIN in HGSOC Precursor Cells.



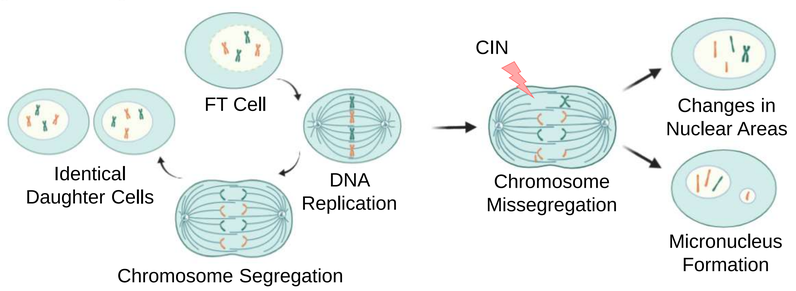
(A) Schematic illustrating a dysplastic precursor lesion at the fimbriated end of the fallopian tube, spreading to the peritoneum and to the ovarian surface epithelium forming a tumor. **(B)** Illustration presenting the SCF complex-mediated poly-ubiquitination and proteasomal degradation of a substrate. The SCF complex consists of four protein subunits: RBX1, SKP1, CUL1 and an F-box protein.

HYPOTHESIS

Hypothesis: Diminished *SKP1* or *RBX1* expression and genotoxic stress will exacerbate CIN in FT cells to ultimately promote disease development.

EVALUATING CIN

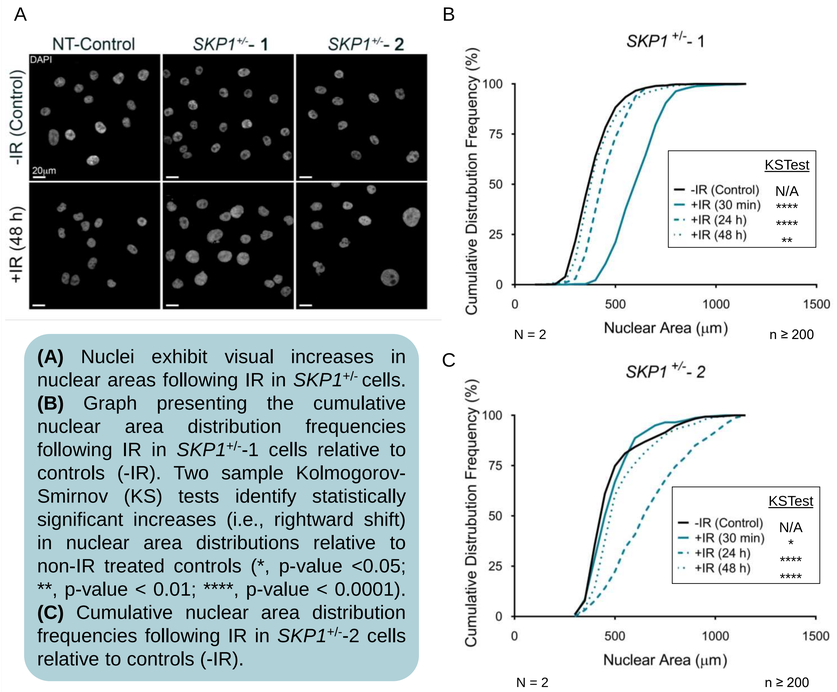
Figure 2. Changes in Nuclear Area and Micronucleus Formation are Indicative of CIN.



Schematic showing how unequal chromosome segregation (*i.e.* CIN) induces increases in micronucleus formation and changes in nuclear areas.

RESULTS

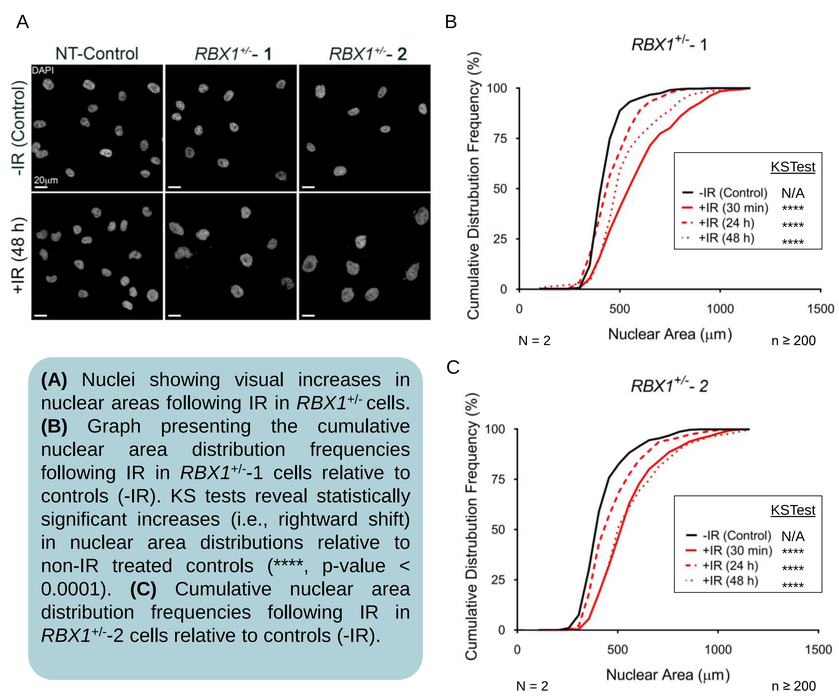
Figure 3. Ionizing Radiation Induces Increases in Nuclear Areas in *SKP1*$^{+/-}$ Clones.



(A) Nuclei exhibit visual increases in nuclear areas following IR in *SKP1*$^{+/-}$ cells. **(B)** Graph presenting the cumulative nuclear area distribution frequencies following IR in *SKP1*$^{+/-}$-1 cells relative to controls (-IR). Two sample Kolmogorov-Smirnov (KS) tests identify statistically significant increases (i.e., rightward shift) in nuclear area distributions relative to non-IR treated controls (*, p-value <0.05; **, p-value < 0.01; ****, p-value < 0.0001). **(C)** Cumulative nuclear area distribution frequencies following IR in *SKP1*$^{+/-}$-2 cells relative to controls (-IR).

RESULTS

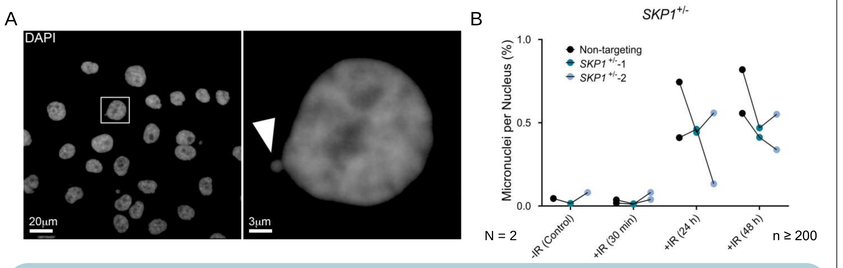
Figure 4. Ionizing Radiation Induces Increases in Nuclear Areas in *RBX1*$^{+/-}$ Clones.



(A) Nuclei showing visual increases in nuclear areas following IR in *RBX1*$^{+/-}$ cells. **(B)** Graph presenting the cumulative nuclear area distribution frequencies following IR in *RBX1*$^{+/-}$-1 cells relative to controls (-IR). KS tests reveal statistically significant increases (i.e., rightward shift) in nuclear area distributions relative to non-IR treated controls (****, p-value < 0.0001). **(C)** Cumulative nuclear area distribution frequencies following IR in *RBX1*$^{+/-}$-2 cells relative to controls (-IR).

RESULTS

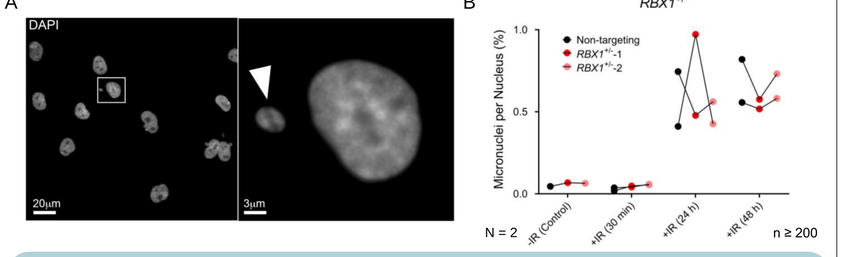
Figure 5. Ionizing Radiation Enhances Micronucleus Formation in *SKP1*$^{+/-}$ Clones.



(A) Representative nuclei showing qualitative changes in micronucleus formation following IR treatment in *SKP1* deficient cells. Bounding box (white) identifies magnified region presented on the right highlighting a micronucleus (arrowhead). **(B)** Dot plot of micronucleus formation following IR in *SKP1*$^{+/-}$-1, *SKP1*$^{+/-}$-2 relative to non-targeting (NT-) control at the indicated timepoints post-IR treatment.

RESULTS

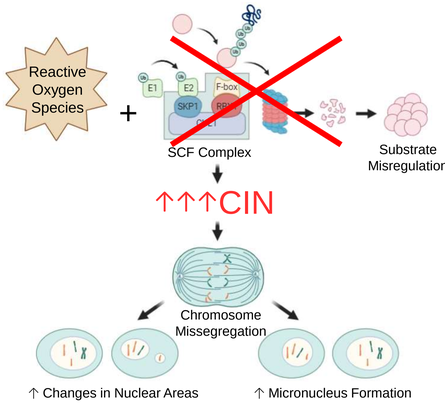
Figure 6. Ionizing Radiation Enhances Micronucleus Formation in *RBX1*$^{+/-}$ Clones.



(A) Representative nuclei showing qualitative changes in micronucleus formation following IR treatment in *RBX1* deficient cells. Bounding box (white) identifies magnified region presented on the right highlighting a micronucleus (arrowhead). **(B)** Dot plot of micronucleus formation following IR in *RBX1*$^{+/-}$-1 and *RBX1*$^{+/-}$-2 relative to NT-control at the indicated timepoints post-IR treatment.

SUMMARY AND CONCLUSION

Figure 7. Ionizing Radiation Exacerbates CIN in *SKP1*$^{+/-}$ and *RBX1*$^{+/-}$ clones.



Summary:

- IR induces significant increases nuclear areas and enhances micro-nucleus formation in *SKP1*$^{+/-}$ and *RBX1*$^{+/-}$ clones.

Conclusion:

- Data shows that IR exacerbates CIN in *SKP1*$^{+/-}$ and *RBX1*$^{+/-}$ clones (illustrated in schematic, left) and supports the possibility that genotoxic stress produced during ovulation may synergize with genetic defects in *SKP1* and *RBX1* to promote HGSOC pathogenesis.

ACKNOWLEDGMENTS

We thank CancerCare Manitoba Foundation for operational support (KJM).