Investigating the Role Aberrant Ubiquitin Regulation has on Chromosome Instability and Colorectal Cancer Pathogenesis

CancerCare Manitoba RESEARCH INSTITUTE
Care through Discovery

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Introduction

Colorectal cancer (CRC) is the 3rd most diagnosed and 2nd most lethal cancer in Canada[1]. Chromosome instability (CIN: increased rate of chromosome gains and losses) is a known driver of CRC that occurs in 85% of cases[2,3]. The molecular determinants driving CRC development and progression and CIN remain largely unknown, but recent data from the McManus laboratory indicates aberrant ubiquitin regulation as a key driver of CIN[4]. Ubiquitylation is the process of adding ubiquitin onto a target protein, and deubiquitylation is the process of removing ubiquitin from a target protein. Ubiquitylation and deubiquitylation are also involved in regulating cellular processes such as protein activity, localization, and degradation, DNA damage response, Mitotic spindle assembly, chromosome segregation, and cell cycle checkpoints. **(Fig. 1)**. Aberrant regulation of these processes have been implicated in inducing CIN[4].

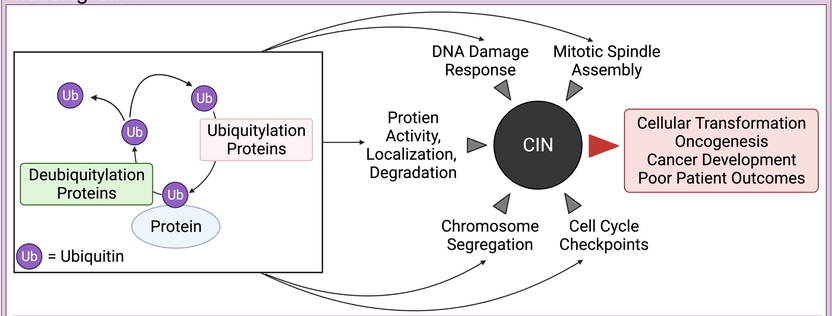

Figure 1. Ubiquitylation and deubiquitylation regulate several cellular processes. Aberrant regulation of these processes can result in CIN, which subsequently induces cellular transformation, oncogenesis, cancer development, and ultimately poor patient outcomes.

Hypothesis

Hypothesis: We hypothesize diminished expression of *USP53* will induce CIN in colorectal models.

Experimental Approach

Table 1. Cellular models employed in the duration of this project.

Cell Line	Description
HCT116	Human colorectal carcinoma (malignant)
1CT	Immortalized human colonic epithelial cell line (non-malignant)
A1309	1CT derivative cell line with additional alterations in *KRAS*, *TP53*, and *APC,* proposed to be early etiological events in CRC (non-malignant)

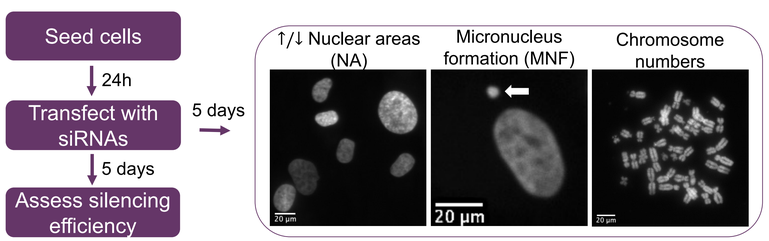

Figure 2. Workflow to evaluate CIN phenotypes. Schematic depicting experimental layout (left) used when conducting CIN assays. CIN assays are performed to assess nuclear area heterogeneity, micronucleus formation (small, DNA-containing body excluded from primary nucleus; white arrow), and changes in chromosome number, all of which are CIN phenotypes.

Results

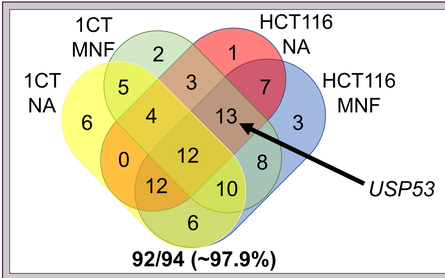

Figure 3. *USP53* is a putative CIN gene in HCT116 and 1CT. An siRNA-based screen of 94 deubiquitylation genes was conducted in HCT116 and 1CT. 92 out of 94 genes induced CIN-associated phenotypes. Silencing of *USP53* induced changes in nuclear area in HCT116 and increases in micronucleus formation in HCT116 and 1CT.

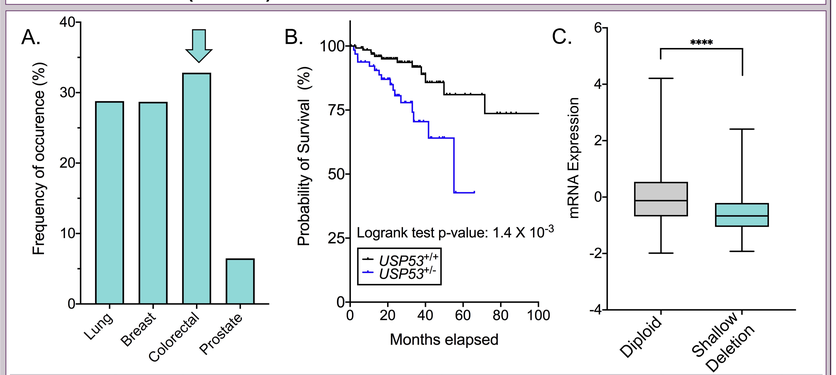

Figure 4. *USP53* is frequently altered in cancer. *(A).* Bar graph presenting the frequency of *USP53* shallow deletion in 4 common cancer types including CRC (arrow). ***(B).*** Kaplan-Meier curve reveals patients with loss of *USP53* have worse disease-free survival compared to those with diploid copies. ***(C).*** Box and whisker plot demonstrates *USP53* copy number loss corresponds to mRNA expression (expression is normalized to normal samples; y-axis values represent standard deviations from mean). Student's t-test reveals significant differences between samples (****, p-value < 0.0001) . All data were extracted from The Cancer Genome Atlas[5] via cBioPortal[6,7].

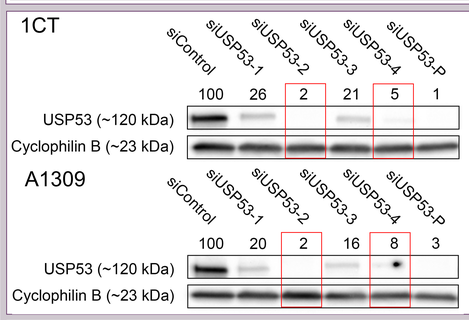

Figure 5. *USP53* is efficiently silenced in 1CT and A1309. Semi-quantitative western blots depicting decreased *USP53* expression relative to siControl following transfection with individual (*USP53*-1, -2, -3, -4) and pooled (*siUSP53*-P; equimolar amounts of siUSP53-1 to -4) siRNAs. USP53 expression is normalized to the loading control (Cyclophilin B). si*USP53*-2 and -4 were identified as the most efficient individual siRNA duplexes which, along with si*USP53*-P, were selected for all subsequent experiments (red boxes).

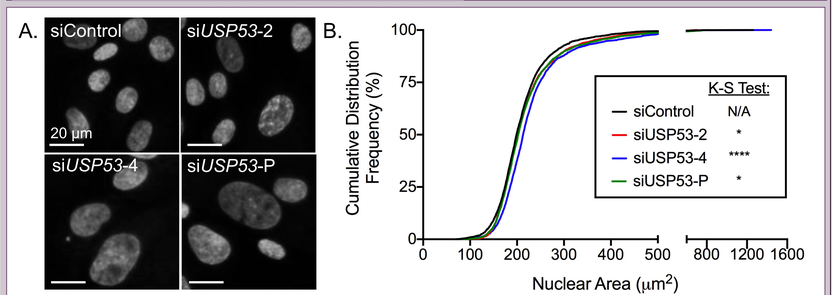

Figure 6. *USP53* silencing induces nuclear area (NA) increases in 1CT. *(A).* Hoescht stained *USP53* silenced nuclei show visual nuclear area increases relative to the negative control (si*Control*). ***(B).*** Cumulative distribution frequency (CDF) plot depicting significant NA increases following *USP53* silencing relative to negative control. Two-Sample Kolmogorov-Smirnov (K-S) test (*, p-value < 0.05; ****, p-value <0.0001), N=1, n=6.

Results

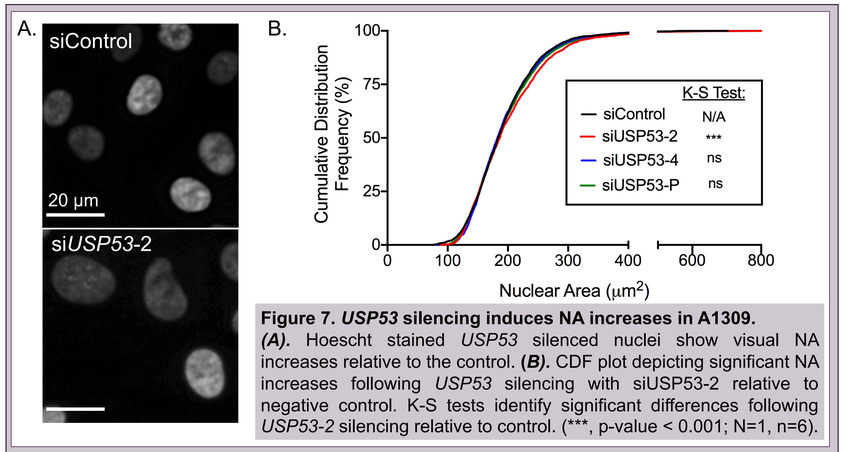

Figure 7. *USP53* silencing induces NA increases in A1309. ***(A).*** Hoescht stained *USP53* silenced nuclei show visual NA increases relative to the control. ***(B).*** CDF plot depicting significant NA increases following *USP53* silencing with siUSP53-2 relative to negative control. K-S tests identify significant differences following *USP53-2* silencing relative to control. (***, p-value < 0.001; N=1, n=6).

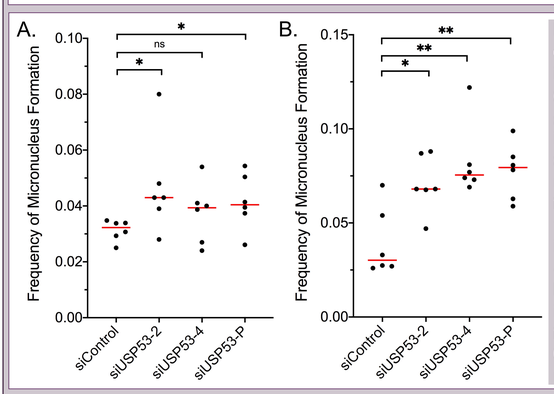

Figure 8. Reduced *USP53* expression induces increases in micronucleus formation (MNF) in 1CT and A1309. *(A).* Dot plot depicting increases in the frequency of MNF (number of micronuclei normalized to number of nuclei) following *USP53* silencing in 1CT. **(B).** Dot plot presenting increases in MNF following *USP53* silencing in A1309. Mann-Whitney (MW) Tests identify significant differences following silencing (*, p-value < 0.05; **, p-value < 0.01; N=1, n=6).

Conclusions and Significance

- *USP53* exhibits shallow deletions in ~33% of CRCs and loss of *USP53* correlates with worse disease-free survival in CRC patients
- Reduced *USP53* expression leads to increases in CIN phenotypes in colorectal cell lines including nuclear area heterogeneity (1CT and A1309) and increases in micronucleus formation (1CT and A1309)
- **Significance**: Reduced *USP53* expression induces CIN, suggesting it may be an early etiological event in colorectal cancer pathogenesis

Future Directions

- Assess reproducibility of CIN assays by conducting more replicates
- Assess changes in chromosome numbers following *USP53* silencing in 1CT + A1309
- Generate knockout models using CRISPR-Cas9
- Evaluate the long-term impacts reduced *USP53* expression have on CIN and cellular transformation

References and Acknowledgments

(1) Canadian Cancer Society Advisory Committee. *CMAJ.* (2020). (2) Geigl, J. *Trends Genet.* (2008) (3) Orr, B., et al. *Front Oncol.* (2013). (4) Jeusset, L. M., et al. *Cancers.* (2021). 13, 1043. (5) Hoadley, K. A., et al. *Cell.* (2018) 173(2), 291. (6) Cerami, E., et al. *Cancer Discov.* (2012). 2(10), 960. (7) Gao, J., et al, *Sci Signal.* (2013). 6(269), 1.

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