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

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Introduction

Colorectal cancer (CRC) is the 3rd most diagnosed and 2nd most lethal cancer in Canada1. Chromosome instability (CIN: increased rate of chromosome gains and losses) is a known driver of CRC that occurs in 85% of cases2,3. 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 CIN4. Ubiquitination is the process of adding ubiquitin onto a target protein, and deubiquitination is the process of removing ubiquitin from a target protein. Ubiquitination and deubiquitination 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 CIN4.

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

Experimental Approach

<table>
<thead>
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<th>Table 1. Cellular models employed in the duration of this project.</th>
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<td><strong>Cell Line</strong></td>
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<td>HCT116</td>
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<tr>
<td>1CT</td>
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<tr>
<td>A1309</td>
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Seed cells

24h

Transfected with

siRNAs

5 days

5 days

Assess silencing

efficiency

1/1 Nuclear areas (NA) | Microtubule formation (MF) | Chromosome numbers

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, microtubule 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 micronuclear 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 copy. (C) Box and whisker plot demonstrates USP53 copy number loss correlates 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 < 0.0001). All data were extracted from The Cancer Genome Atlas via cBioPortal.

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) siRNA. USP53 expression is normalized to the loading control (Cyclophilin B). siUSP53-2 and -4 were identified as the most efficient individual siRNA duplexes which, along with siUSP53-P, were selected for all subsequent experiments (red boxes).

Figure 6. USP53 silencing induces nuclear area (NA) increases in 1CT. (A) Hoechst stained USP53 silenced nuclei show visual nuclear area increases relative to the negative control (siControl). (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 < 0.05; **p < 0.001; ***p < 0.0001). N=15, mb.

Figure 7. USP53 silencing induces NA increases in A1309. (A) Hoechst 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 test identifies significant differences following USP53-2 silencing relative to control. (***p < 0.001; N=15, mb).

Figure 8. Reduced USP53 expression induces increases in micronuclear formation (MFN) in 1CT and A1309. (A) Dot plot depicting increases in the frequency of MFN (number of micronuclei normalized to number of nuclei) following USP53 silencing in 1CT. (B) Dot plot presenting increases in MFN following USP53 silencing in A1309. Mann-Whitney (MW) tests identify significant differences following silencing (*p < 0.05; **p < 0.01; ***p < 0.001).

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 micronuclear 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 CRC and cellular transformation.

References and Acknowledgments


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