Investigating the Impacts Reduced USP4 has on Chromosome Instability and its Implications in Colorectal Cancer

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**Introduction and Rationale**

Colorectal cancer is one of the leading cancers diagnosed and one of the deadliest cancers in Canada – accounting for ~12% of new cancer cases and causing 9,700 deaths in 2020\(^4\). With these devastating statistics, it is imperative to further our understanding of the underlying genes, proteins and pathways driving cancer development and progression. Chromosome instability (CIN) is a hallmark of genomic instability, defined as an increase in the rate at which whole chromosomes or chromosome fragments are gained or lost\(^4\). CIN is associated with aggressive tumours, metastasis and poor patient prognosis, driving cancer development and progression in ~85% of colorectal cancers\(^4,5\), yet the molecular alterations driving CIN remain largely uncharacterized.

Recent findings from the McManus laboratory suggest reduced expression of genes regulating ubiquitin dynamics may be key drivers of CIN. Preliminary data from an siRNA knockdown screen of ~700 ubiquitination/deubiquitination genes identified USP4 (Ubiquitin-specific peptidase 4) as a putative driver of CIN. USP4 encodes a deubiquitinating enzyme involved in various cellular functions, including Wnt/catenin signalling, DNA damage repair, chromosome segregation and mRNA splicing\(^6\). Clinical patient data highlight the clinical relevance of USP4 in colorectal cancer, as it is heterozygously lost in ~16% of colorectal cancers and is associated with worse patient outcomes (Figure 1\(^\text{A}\)). Here, we investigated the impact reduced USP4 expression has on the development of CIN and colorectal cancer through assessment of CIN phenotypes in two non-malignant colonic epithelial cell lines, 1CT and 1CT-derived, A1309.

**Materials and Methods**

**Figure 1.** USP4 is frequently lost in colorectal cancer and associated with worse patient survival

**Figure 2.** Experimental approaches used to evaluate CIN phenotypes

**Figure 3.** USP4 protein expression is effectively reduced following siRNA-based silencing

**Figure 4.** Diminished USP4 expression induces changes in nuclear areas

**Figure 5.** Micronucleus formation is increased following USP4 silencing

**Figure 6.** USP4 silencing synergizes with genetic defects in 1A309 to exacerbate increases in nuclear area distributions.

**Figure 7.** USP4 silencing induces aberrant chromosome phenotypes

**Conclusion and Significance**

- Diminished USP4 expression induces CIN in 1CT and A1309, with increases in nuclear area heterogeneity, increases in micronuclear formation, and trends increasing in aberrant chromosome phenotypes.
- CIN phenotypes in A1309 compared to 1CT, namely more extreme shifts in nuclear area distributions (increases in nuclear area heterogeneity suggest large-scale chromosome gains/losses) highlight potential synergy between diminished USP4 and alterations associated with colorectal cancer development (KRAS, TP53, and APC).
- These findings identify USP4 as a driver of CIN in a colorectal epithelial context, which may have implications in colorectal cancer development and pathogenesis.

**Future Directions**

- Generation and validation of USP4 gene knockouts in 1CT and A1309
- Assess impacts on chromosome numbers, dynamics of CIN over time
- Roles in development of cancer-associated characteristics (cellular transformation)

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