Evaluating SKP2 as a Chromosome Instability Gene in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is the 3rd most commonly diagnosed, and 2nd most lethal cancer among Canadians1. Many CRC diagnoses occur in late stages (III or IV)2, but molecular determinants (i.e. abnormal genes and cellular pathways) giving rise to CRC remain poorly understood. Chromosome instability (CIN) is a form of genome instability suspected to drive CRC. CIN is defined by ongoing, progressive changes in losses or gains of whole chromosome or large fragments, associated with ~85% of CRCs2. CIN induces cell-cell heterogeneity, leading to selective advantages or disadvantages, ultimately leading to tumor evolution, metastasis, and overall poor patient prognosis3. It is essential to investigate and characterize the origins and early events contributing to CRC development and progression for new therapies.

Aim 1: Determine the clinical relevance of reduced SKP2 expression in CRC

Figure 6. A. SKP2 is frequently altered in many cancer types, including CRC3. HomDel (deep blue)= Homozygous deletions (deep deletions), Amp (red)=amplification, HetLoss (blue)= heterozygous loss (shallow deletions). Gains (green)= gains and changes in chromosome numbers. Figure created using Biorender.

Results

Aim 2: Evaluate the impact reduced SKP2 expression from silencing has on CIN phenotypes in CRC cell lines

Figure 7. Semi-quantitative Western Blots demonstrating the effectiveness of SKP2 silencing in A1309 (left) and HCT116 (right) cells. The semi-quantitative analyses in both A1309 and HCT116 compare expression levels of SKP2 tosiControl following silencing. Minimum of 1000 nuclei/condition x 6 replicate wells analyzed per condition, with N=3. Statistical significance is (shallow deletions).

Figure 8. Cumulative Distribution Frequency Histograms of Nuclear Areas (NA) from A1309 (left) and HCT116 (right). The Kolmogorov-Smirnov (KS) test compares cumulative nuclear area distributions relative to siControl (not applicable to A1309). In A1309, there is a significant rightward shift following treatment compared to siControl. In HCT116, there is a significant rightward shift following treatment compared to siControl.

Conclusion and Significance

• SKP2 exhibits copy number losses in many cancers (CRC). Copy number losses correspond with significantly reduced expression (mRNA). Reduced expression correlates with worse patient survival.

• Diminished SKP2 expression can induce chromosome instability (CIN) phenotypes in both HCT116 and A1309 epithelial colorectal cancer cell lines.

• SKP2 silencing leads to significant increases in nuclear area heterogeneity, and micronucleus formation in A1309.

• SKP2 silencing leads to significant increases in nuclear area heterogeneity in HCT116.

• These data support the possibility that reduced SKP2 expression may contribute to CRC pathogenesis.

Future Directions

• skiSKP2 mitotic chromosome spreads enumerated to evaluate changes in chromosome compliments for karyotypic heterogeneity

• Use CRISPR/Cas9 approaches to develop clinically relevant SKP2 +/- clones and assess the long-term impact on CIN, karyotypic evolution and cellular transformation (i.e. early disease development)

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