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 Canadians[1]. Many CRC diagnoses occur in late stages (III or IV)[1], 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 CRCs[2]. CIN induces cell-cell heterogeneity, leading to selective advantages or disadvantages, ultimately leading to tumor evolution, metastasis, and overall poor patient prognosis[3]. It is essential to investigate and characterize the origins and early events contributing to CRC development and progression for new therapies[3].

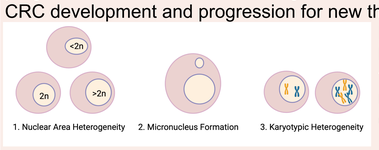


Figure 1. Three hallmarks of CIN include nuclear area heterogeneity (changes in DNA content), micronucleus formation (extra nuclear bodies separate from primary nucleus), and karyotypic heterogeneity (changes in chromosome numbers). Figure created using Biorender.

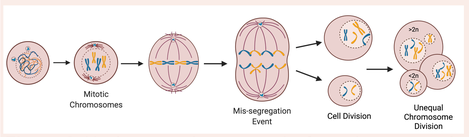


Figure 2. Nuclear area heterogeneity from chromosomal missegregation event involving uneven cell division. The result is unequal chromosome distribution among daughter cells, with potential advantages or disadvantages from heterogeneity. Figure created using Biorender.

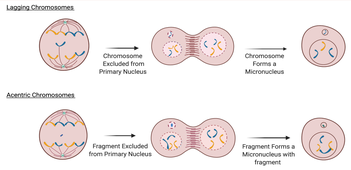


Figure 3. Micronucleus formation arising from abnormal chromosome divisions create lagging or acentric chromosomes from improper mitotic spindle retraction. Figure created using Biorender.

SKP2, an F-box encoding gene within the *SKP1*, *CUL1*, and F-box protein (SCF) complex, has been identified as a potential CIN gene. The function of SKP2 as an F-box protein provides specificity for its substrates Cyclin E1, Cyclin E2, and p27^{kip1} to be recognized for ubiquitination, and subsequent degradation by the 26S proteasome[4]. Abnormal SCF complex function can lead to substrate accumulation resulting in CIN- associated phenotypes[4].

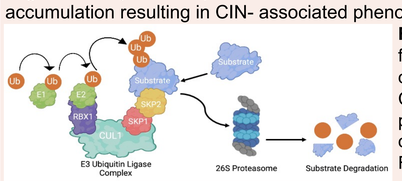


Figure 4. SCF complex schematic depicting normal functions of polyubiquitination followed by proteasomal degradation of substrates (SKP2 targets Cyclin E1, Cyclin E2, p27^{kip1}). Protein substrate binds to F-box protein inducing ubiquitination and subsequent degradation by 26S proteasome. Ub=Ubiquitylated. Figure created using Biorender.

Hypothesis and Research Aims

Hypothesis: Decreased *SKP2* expression induces CIN that promotes cellular transformation and contributes to CRC progression.

Aim 1- Determine the clinical relevance of reduced *SKP2* expression in CRC.

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

Experimental Approach

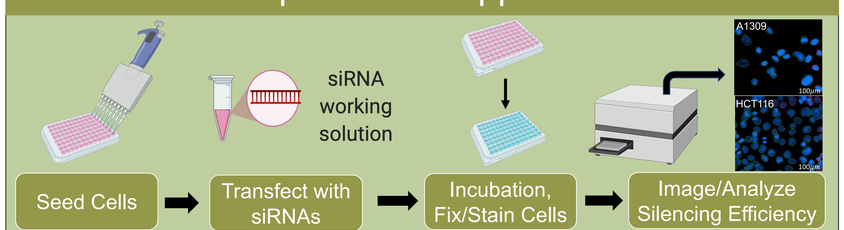
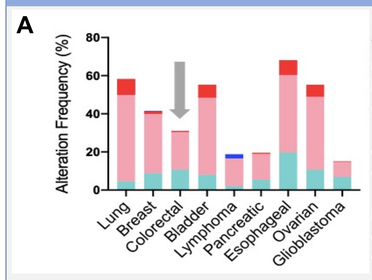


Figure 5. CIN assays used to evaluate changes in CIN-associated phenotypes including nuclear area heterogeneity, and micronucleus formation. Cells were seeded, then transfected with siRNAs targeting SKP2 of siControl 24 hours later. Cells were permitted to grow (4 days), at which point they are fixed (paraformaldehyde), and counterstained (Hoechst; nuclear stain). On day 5 plate was imaged and qualitatively assessed for changes in nuclear area and micronucleus formation relative to siControl.

Results

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



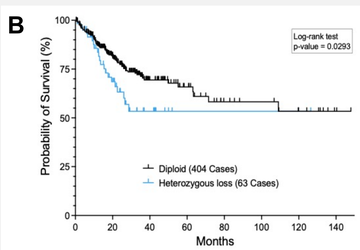


Figure 6. A. *SKP2* is frequently altered in many cancer types, including CRC[5]. HomDel (deep blue)= Homozygous deletions (deep deletions), Amp (red)=amplification, Hetloss (blue)= heterozygous loss (shallow deletions). Gains (pink).

Figure 6. B. *SKP2* copy number losses are associated with decreased overall survival in CRC patients compared to diploid cases[5].

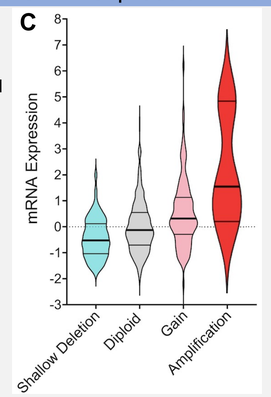


Figure 6. C. mRNA expression of *SKP2* relative to diploid. Decreased mRNA expression=less copy numbers, increased mRNA expression=more copy numbers compared to diploid. Data from cBio Portal database[5].

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

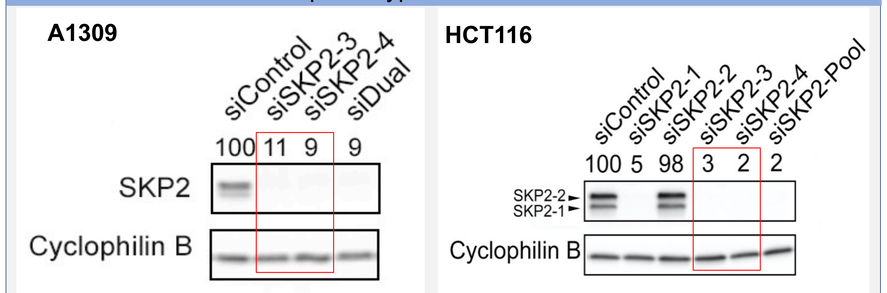


Figure 7. Semi-quantitative Western Blots demonstrating the effectiveness of *SKP2* silencing in A1309 (left) and HCT116 (right) cells. The semi-quantitative analyses in both A1309 and HCT116 compare samples that are normalized to the respective loading control (Cyclophilin B) and are presented relative to siControl. In HCT116, two isoforms of SKP2 (SKP2-1 and SKP2-2) are shown.

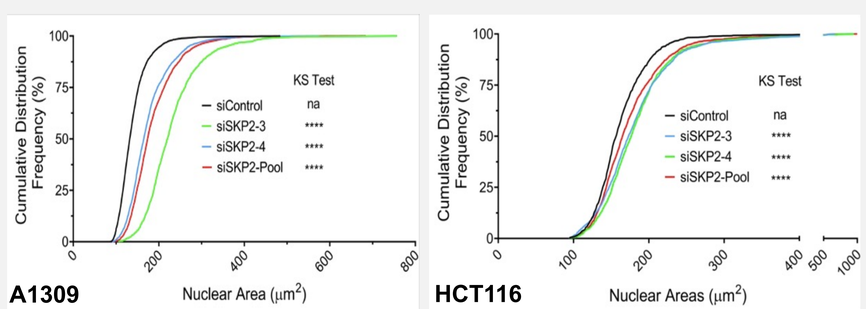


Figure 8. Cumulative Distribution Frequency Histograms of Nuclear Areas (NA) from A1309 (left) and HCT116 (right). The Kolmogorov-Smirnov (KS) test compares cumulative nuclear area distributions relative to siControl (na=not applicable, **** p-value <0.0001). In A1309, there is a significant rightward shift following treatment compared to siControl. In HCT116, there is a significant rightward shift following treatment compared to siControl.

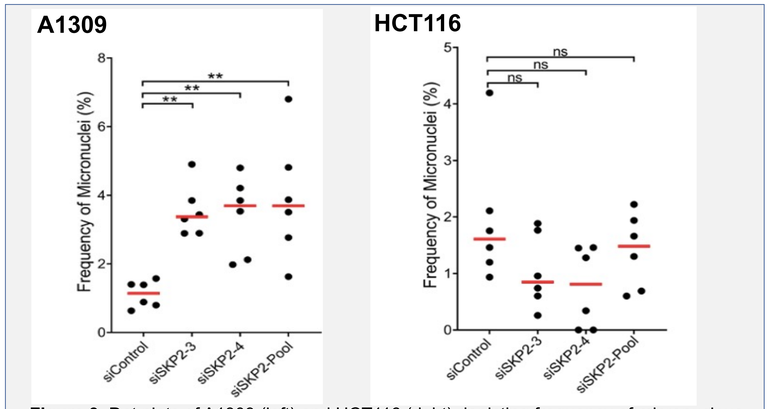


Figure 9. Dot plots of A1309 (left) and HCT116 (right) depicting frequency of micronucleus formations following *SKP2* silencing relative to non-targeting controls. Red bar indicates the median of 6 replicate values. Statistical analysis is indicated as non-significant (ns) or **, p-value <0.01 using the Mann-Whitney (MW) test. In A1309, a minimum of 1000 nuclei/condition x 6 replicate well analyzed per condition, with N=3. Statistical significance is achieved in A1309, demonstrating an increase in micronucleus formations compared to siControl following *SKP2* silencing. Minimum of 1000 nuclei/condition x 6 replicate wells analyzed per condition, N=1 in HCT116. There are not significant changes in micronucleus formations following silencing of *SKP2* in HCT116.

Conclusions and Significance

- *SKP2* exhibits copy number losses in many cancers (CRC). Copy number losses correspond with significantly reduced expression (mRNA). Reduced expression correlated 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

- siSKP2 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)

Acknowledgements/References

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