Exploring FBXO7 as a Candidate Chromosome Instability Gene in Colorectal Cancer

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INTRODUCTION
Colorectal Cancer (CRC) is the 3rd most diagnosed the 2nd most lethal cancer in Canadians. A better understanding of the genes that play a role in CRC tumour progression is needed for the creation of new therapeutic strategies. Chromosome Instability (CIN) occurs in 80-85% of CRCs. CIN is associated with all tumour types and is characterized by an increase in rate at which whole chromosomes or large chromosomal fragments are gained or lost. CIN typically involves changes in chromosome complements (DNA content), which leads to changes in nuclear area, and micronuclei formation (MNF), which are surrogate markers for CIN. CIN induces cell-to-cell heterogeneity, which leads to selective growth advantages in cancer cells, disease progression, aggressive disease, multi-drug resistant tumours, and overall poor patient prognosis.

AIM 1: Investigate FBXO7 mutations in various cancer types
- Using cancer genome databases (Chro Portal and Cosmic)
- Evaluate impact of findings using PolyPhen

AIM 2: Determine chromosome phenotypes in HCT116, A1309 following FBXO7 silencing
- Image software used to view chromosome images

AIM 3: Generate and validate FBXO7-knockout clones in A1309 cell line
- Guide strand + Cas9 used to knockout FBXO7
- Limited dilutions done to yield clonal populations of FBXO7 knockout clones
- Validation of knockout clones using Western Blot and DNA Sequencing analyses

METHODS

RESULTS

Figure 4. A. FBXO7 is frequently altered in many cancer types, including CRC (grey arrow). B. Copy number loss of FBXO7 is associated with decreased overall survival in CRC patients. C. Decreased FBXO7 mRNA expression is associated with decreased overall survival in CRC patients.

Figure 5. Illustration presenting key functional domains and binding motifs of isoform 1 of FBXO7.

Figure 6. Chart depicting frequency of FBXO7 mutations in patient samples (n=214).

Figure 7. Bar graph depicting frequency of impact missense substitution mutations evaluated by PolyPhen (n=14).

Figure 8. Amino Acid sequence of full length FBXO7 protein. B. Amino Acid sequence of successful heterozygous knockout of FBXO7, introducing a premature stop codon in one allele. C. Amino Acid sequence of successful homoygous knockout of FBXO7, introducing a premature stop codon in both alleles.

Figure 9. Increases in chromosome aberrations and abnormal phenotypes correspond with FBXO7 silencing. A. Dot plot depicting percentage of defects in spreads of three biological replicates in HCT116 with median indicated (red bar). Statistical difference between control and siFBXO7-4 (t-test, * p-value < 0.05). B. Dot plot depicting percentage of defects in spreads of three biological replicates in HCT116 with median indicated (red bar). Statistical difference between control and siFBXO7-4 (t-test, * p-value < 0.05). General increasing trend is also observed in other conditions (t-test, n= p-value > 0.05). C. Dot plot depicting frequency of fragmentation defects in spreads of three biological replicates in HCT116 with median indicated (red bar). Although statistical significance was not achieved (t-test, n= p-value > 0.05), a general trend of increasing aberrations in silenced conditions is observed. D. Dot plot depicting frequency of single chromatid breaks in spreads of three biological replicates in HCT116 with median indicated (red bar). Although statistical significance was not achieved (t-test, n= p-value > 0.05), a general trend of increasing aberrations in silenced conditions is observed.

CONCLUSION
- FBXO7 mutations are found in every functional domain of the protein
- FBXO7 mutations show the potential to be damaging
- Decreased FBXO7 expression results in increased frequency of chromosome aberrations in HCT116 and A1309
- FBXO7 can be heterozygously and homoygously knocked out in A1309

FUTURE DIRECTIONS
- Assess CIN and cellular transformation in FBXO7-knockout clones over time
- Using knockout clones in mouse models to establish ability of knockouts to form tumours.

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REFERENCES