Exploring *FBXO7* as a Candidate Chromosome Instability Gene in Colorectal Cancer

Tooba Razi(1), Michaela C.L. Palmer(2,3), Kirk J. McManus(2,3)

Faculty of Science, University of Manitoba(1), Department of Biochemistry and Medical Genetics, University of Manitoba(2); Research Institute in Oncology and Hematology(3), Winnipeg, MB, Canada

INTRODUCTION

Colorectal Cancer (CRC) is the 3rd most diagnosed the 2nd most lethal cancer in Canadians[1]. 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[2]. 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[2]. 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[4].

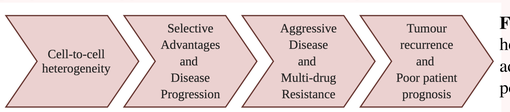

Figure 1. CIN and cell-to-cell heterogeneity provide selective growth advantages to cancer cells leading to poor patient prognosis.

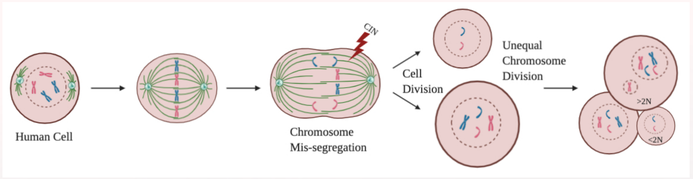

Figure 2. CIN causes unequal chromosome division in cells, leading to changes in nuclear area, changes in nuclear DNA content, and formation of micronuclei.

Recent preliminary data has identified *FBXO7,* an F-box encoding gene, as a novel CIN gene. FBXO7's function in the Skp1-Cul1-Fbox (SCF)-complex is its most well studied role, in which it binds to a substrate so it may be recognized to be ubiquitinated, and subsequently degraded by the 26S proteosome[3]. Malfunction of the SCF complex can lead to accumulation of substrate, resulting in CIN-associated phenotypes. *FBXO7* is frequently altered in many cancers. Additionally, gene copy number loss and decreased mRNA expression has been associated with poor patient outcomes.

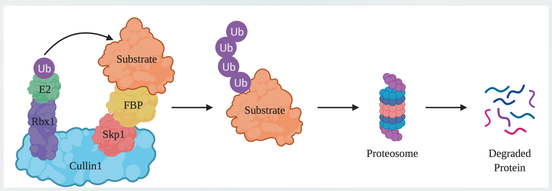

Figure 3. Schematic depicting normal SCF complex function. Protein substrate binds to F-box protein, which is then ubiquitinated, leading to degradation by 26S proteosome.

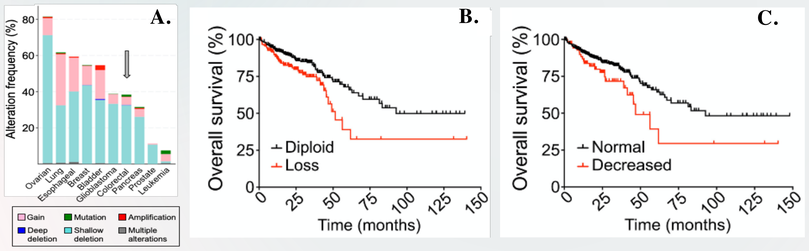

Figure 4. A. *FBXO7* is frequently altered in many cancer types, including CRC (grey arrow). **B**. Copy number loss of *FBXO7* is associated with decreased overall survival in CRC patients. **C**. Decreased *FBXO7* mRNA expression is associated with decreased overall survival in CRC patients.

PRELIMINARY DATA

- *FBXO7* is effectively silenced in HCT116, A1309, RPA, 1CT
- Reduced FBXO7 expression causes increases in MNF, increases in nuclear area, and changes in chromosome copy number in HCT116, A1309, RPA, 1CT

HYPOTHESIS & RESEARCH AIMS

Hypothesis: FBXO7 is found frequently mutated in cancer, and reduced *FBXO7* expression induces CIN that promotes cellular transformation and contributes to CRC progression.

Aim 1: Investigate mutations associated with cancers that occur in FBXO7

Aim 2: Classify chromosome phenotypes in colonic cell lines following *FBXO7* silencing

Aim 3: Generate and validate *FBXO7*-knockout clones in A1309

FBXO7 1° PROTEIN STRUCTURE

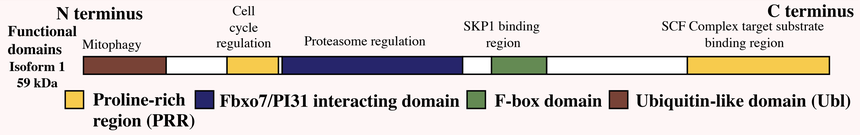

Figure 5. Illustration presenting key functional domains and binding motifs of isoform 1 of FBXO7.

METHODS

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

AIM 2: Determine chromosome phenotypes in HCT116, A1309 following *FBXO7* silencing
- ImageJ 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

RESULTS

AIM 1: Investigate mutations associated with cancers that occur in FBXO7

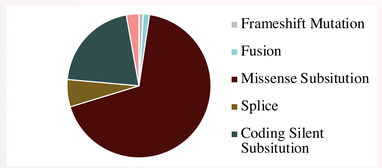

Figure 6. Chart depicting frequency of FBXO7 mutations in patient samples (n=214).

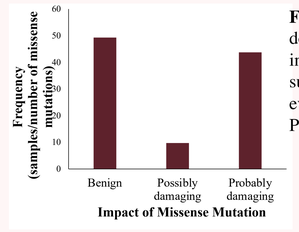

Figure 7. Bar graph depicting frequency of impact missense substitution mutations evaluated by PolyPhen (n=144).

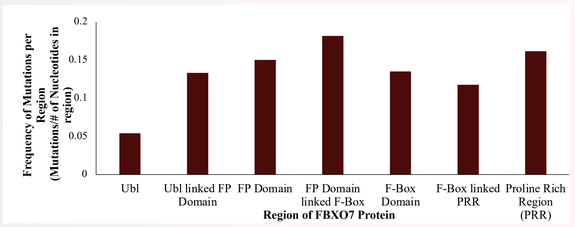

Figure 8. Frequencies of mutations per domain of FBXO7 protein. Data is normalized to the number of nucleotides per region of protein (n=214).

AIM 2: Classify chromosome phenotypes in HCT116 following *FBXO7* silencing

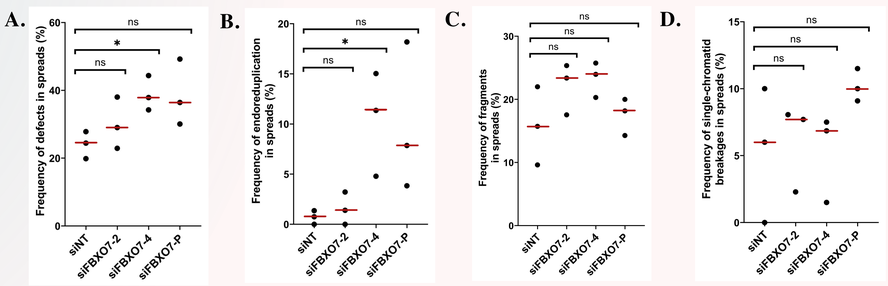

Figure 9. Increases in chromosome aberrations and abnormal phenotypes correspond with *FBXO7* silencing. **A**. Dot plot depicting percentage of defects in spreads of three biological replicates in HCT116 with median indicated (red bar). Statistical difference between control and siFBXO7-4 (t-test, *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

RESULTS

AIM 2: Classify chromosome phenotypes in A1309 following FBX07 silencing

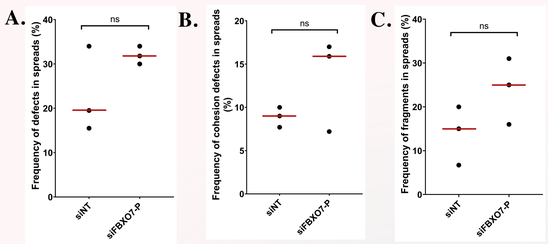

Figure 10. Dot plots depicting frequency of defects in spreads (A), cohesion defects (B), and fragmentation (C) in spreads of three replicates in A1309 with median indicated (red bar). Although statistical significance was not achieved (t-test, ns = p-value > 0.05), a general trend of increasing aberrations in silenced conditions is observed.

AIM 3: Generate and Validate knockout clones of FBXO7 in A1309

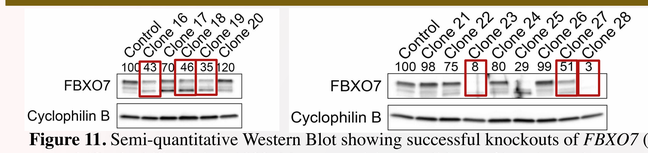

Figure 11. Semi-quantitative Western Blot showing successful knockouts of *FBXO7* (red boxes) against control (Cyclophilin B) in A1309. Many promising candidates of knockout clones were identified for sequencing.

A.

Full-length FBXO7 Protein
MRLRVRLLKRTWPLEVPETEPTLGHLRSHLRQSLLCTWGYSSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILQDDIPAPNIPSSTDSEHSSLQNNEQPSLATSSNQTSMQD
EQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQDNAHMAEGTGFYPSEPMLCSESVEGQVPHSLETLYQSADCSDANDALIVLHLLMLESGYIPQGTEAKALSMPEKWK
LSGVYKLQYMHPLCEGSSATLTCVPLGNLIVVNATLKINNEIRSVKRLQLLPESFICKEKLGENVANIYKDLQKLSRLFKDQLVYPLLAFTRQALNLPDVFGLVVLPLELKLRIFR
LLDVRSVLSLSAVCRDLFTASNDPLLWRFLYLRDFRDNTVRVQDTDWKELYRKRHIQRKESPKGRFVMLLPSSTHTIPFYPNPLHPRPFPSSRLPPGIIGGEYDQRPTLPYVGDPIS
SLIPGPGETPSQFPPLRPRFDPVGPLPGPNPILPGRGGPNDRFPFRPSRGRPTDGRLSFM

B.

Clone 1:
Allele 1:
MRLRVRLLKRTWPLEVPETEPTLGHLRSHLRQSLLCTWGYSSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILQDDIPAPNIPSSTDSEHSSLQNNEQPSLATSSNQTSMQD
EQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQDNAHMAEGTGFYPSEPMLCSESVEGQVPHSLETLYQSADCSDANDALIVLHLLMLESGYIPQGTEAKALSMPEKWK
LSGVYKLQYMHPLCEGSSATLTCVPLGNLIVVNATLKINNEIRSVKRLQLLPESFICKEKLGENVANIYKDLQKLSRLFKDQLVYPLLAFTRQALNLPDVFGLVVLPLELKLRIFR
LLDVRSVLSLSAVCRDLFTASNDPLLWRFLYLRDFRDNTVRVQDTDWKELYRKRHIQRKESPKGRFVMLLPSSTHTIPFYPNPLHPRPFPSSRLPPGIIGGEYDQRPTLPYVGDPIS
SLIPGPGETPSQFPPLRPRFDPVGPLPGPNPILPGRGGPNDRFPFRPSRGRPTDGRLSFMSTOP
Allele 2:
MRLRVRLLKRTWPLEVPETEPTLGHLRSHLRQSLLCTWGYSSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILQDDIPAPNIPSSTDSEHSSLQNNEQPSLATSSNQTSMQD
EQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQDNAHMAEGTGFYPSEPMLCSESVEGQVPHSLETLYQLTVLMPMMPSTOP

C.

Clone 2:
Allele 1
MRLRVRLLKRTWPLEVPETEPTLGHLRSHLRQSLLCTWGYSSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILQDDIPAPNIPSSTDSEHSSLQNNEQPSLATSSNQTSMQD
EQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQDNAHMAEGTGFYPSEPMLCSESVEGQVPHSLETLSISSTOP
Allele 2
MRLRVRLLKRTWPLEVPETEPTLGHLRSHLRQSLLCTWGYSSNTRFTITLNYKDPLTGDEETLASYGIVSGDLICLILQDDIPAPNIPSSTDSEHSSLQNNEQPSLATSSNQTSMQD
EQPSDSFQGQAAQSGVWNDDSMLGPSQNFEAESIQDNAHMAEGTGFYPSEPMLCSESVEGQVPHSLETLYQLTVLMPMMPSTOP

Figure 12. *FBXO7* can successfully be knocked out in A1309. **A.** 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 homozygous knockout of *FBXO7*, introducing a premature stop codon in both alleles.

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 homozygously knocked out in A1309

Current findings suggest that *FBXO7* is a CIN gene, but further studies must be done to assess the long-term cellular transformation of cells following knockout of *FBXO7*.

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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