**Specific Aims**

Nijmegen breakage syndrome (NBS) is an autosomal recessive disorder characterized by small head size, distinctive facial features, intellectual disability, short stature, immunodeficiency, and cancer predisposition1. Less obvious, however, are the unstable chromosomes, accelerated shortening of telomeres, sensitivity to DNA-damaging agents (such as ionizing radiation), and abnormal cell cycle checkpoints experienced by these patients2. Mutations in nibrin (NBN), a gene known for its role in eukaryotic DNA-damage repair, are responsible for these molecular phenotypes. NBN mediates protein-protein interactions at sites of DNA breakage, but lacks any enzymatic activity of its own. However, NBN forms a complex called MRN, with MRE11 and RAD50, which senses DNA double-strand breaks (DSB) and mediates DSB-related cell cycle checkpoint signaling and telomere maintenance3. Nbs1\_C, an NBN domain located at the C-terminus of the protein, is responsible for interaction with MRE11 and ultimately the formation of MRN4. Because of this, NBN can be considered a tumor suppressor protein, for, without this process, cells can accumulate errors that may lead to cell death or uncontrolled growth, offering an explanation for the predisposition to cancer seen in NBS patients.

Mutations in NBN generally lead to a truncated protein. At least eleven mutations are directly linked to NBS. 90% of NBS patients have a 5-nucleotide deletion in the sixth exon, creating a premature stop codon. This mutation, 657del5, results in a fragmented version of NBN, called p26, which lacks the Nbs1\_C domain. However, the remainder of the gene, post p26, is also translated and results in a complementary fragment, called p70, which is essential for viability. The levels of expression of this protein fragment are negatively correlated with cancer predisposition and other NBS phenotypes5.

Additionally, recent research has shown that NBS also affects heterozygotes. These patients experience higher rates of spontaneous and induced chromosome instability and predisposition to malignancies, suggesting that expression of one unaffected gene is not sufficient for efficient DNA-damage repair6. *However*, *the molecular mechanisms that regulate NBN expression in wild type, heterozygotes and mutants, such as 657del5, remain unclear.*

**We will test the hypothesis that the expression levels of nibrin are critical indicators of cancer susceptibility.** Naked mole-rats, a rodent found to possess a very low predisposition to cancer, would pose as great model organisms for studying cancer associated with NBN7. Our ***primary goal*** is to understand the molecular mechanism behind the regulation of NBN expression in these patients.

***Aim 1.*** Identify potential genomic sites affecting NBN expression levels. **Approach**. Perform next generation sequencing on a population of individuals and compare the conservation against the expression levels of NBN. **Hypothesis.** Individuals with high expression of NBN will have shared single nucleotide polymorphisms (SNPs) that indicate potential regulatory sequences for NBN expression. **Rationale.** If these sites affect NBN expression, they will affect the organism’s cancer susceptibility. This information would suggest that manipulation of these genomic regulatory sites could increase the expression of NBN and therefore lower cancer susceptibility in organisms.

***Aim 2.*** Identify NBN expression levels and protein interactions in naked mole-rats compared to humans. **Approach**. Perform quantitative mass spectrometry and compare levels of NBN between these two species. Additionally, identify NBN interaction partners using tandem affinity purification and mass spectrometry. Gene ontology (GO) analysis will be used to compare interactors in naked mole-rats and humans. **Hypothesis.** Average expression levels will be higher in naked mole-rats and the NBN protein in these organisms may possess additional interaction partners. GO analysis will report additional DNA damage responses along with molecular functions involving any new found interaction partners. **Rationale.** Higher expression levels of NBN in naked mole-rats would support the hypothesis that expression levels are critical indicators of cancer susceptibility. Additional interaction partners of NBN in naked mole-rats may pose as potential targets for influencing NBN expression and ultimately lowering cancer susceptibility in other organisms.

***Aim 3.*** Identify potential regulators of NBN expression that may influence cancer susceptibility. **Approach*.*** Conduct a CRISPR-based screen to look for regulators of NBN expression. Once found, the CRISPR-Cas9 system will be used to edit the mouse genome to induce mutations in these putative NBN regulators. We will then analyze NBN expression levels and cancer susceptibility in these mutant mice. **Hypothesis*.*** Mice will possess regulators that affect NBN expression and therefore organism cancer susceptibility. **Rationale*.*** If key regulators of NBN are identified, we can then turn our focus to identifying small molecules and drug options to manipulate their function, ultimately manipulating NBN expression. Increasing NBN expression in an organism will lower NBN related cancer susceptibility.

We seek to understand the regulation of NBN expression at a genomic and proteomic level. With knowledge gained from this research, we pursue a ***long-term goal*** of helping those affected by NBS by providing information that may lead to new treatment options, such as gene therapy and drug developments.

References:

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