Letter of Intent

Project Title: Characterization of lung adenocarcinoma associated mutational hotspot of RAD50 in DNA double strand break repair.

I am an early career researcher in the department of Biochemistry and Microbiology at the University of Victoria, Canada. I am writing to express my strong interest in pursuing this research project focused on **elucidating the role of the cancer-associated rad50-D69Y mutation in DNA double-strand break (DSB) repair pathway** choice. My academic training and research experience in molecular biology, biochemistry and yeast genetics, along with a deep interest in genome stability mechanisms, have prepared me to rigorously address the central hypothesis that Rad50-D69Y alters the balance of repair toward error-prone end joining (EJ), with implications for understanding cancer-associated genomic instability.

Double-strand breaks are among the most deleterious forms of DNA damage, and their repair is tightly regulated through multiple conserved pathways. The Mre11-Rad50-Xrs2/Nbs1 (MRX/N) complex, and particularly the Rad50 subunit, sits at the core of this decision-making process, integrating structural and enzymatic functions to guide the cell toward either homologous recombination (HR) or EJ. Mutations in Rad50, notably the D69Y variant identified in lung adenocarcinoma, may bias repair outcomes and confer survival advantages to cancer cells through increased usage of mutagenic pathways such as alternative end joining (alt-EJ). Understanding the molecular basis of such pathway shifts is critical for both fundamental biology encompassing etiology of lung cancer and the development of targeted therapies.

This project leverages the power of molecular biology, biochemistry and yeast genetics with established DSB repair assays to dissect the functional consequences of rad50-D69Y in vivo. Preliminary data suggest that while rad50-D69Y supports MRX/N complex formation and DNA binding, it compromises key activities such as ATP binding, Tel1/ATM activation, and end resection which are hallmarks of error-free HR-mediated repair. Strikingly, this mutation appears to enhance repair through error-prone EJ, as evidenced by improved growth in an EJ-only strain background and decreased resection efficiency. These findings lay a strong foundation for the proposed investigation into how Rad50-D69Y disrupts repair pathway choice.

The project is thoughtfully designed to test this hypothesis through a series of complementary approaches: (1) quantitative assessment of DSB end-tethering using tagged DNA loci; (2) precise measurement of short- and long-range end resection using qPCR-based strategies; (3) chromatin immunoprecipitation (ChIP-qPCR) to determine the recruitment dynamics of HR and EJ factors; (4) analysis of repair kinetics and mutation signatures through time-course experiments and sequencing. Together, these studies aim to define how the rad50-D69Y mutation perturbs the repair landscape at a mechanistic level.

Sincerely,

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Project proposal

I. Objective

Genomic instability is a hallmark of cancer. Accurate double-strand break (DSB) repair prevents chromosomal rearrangements that drive tumorigenesis. The objective of this project is to define **how the lung adenocarcinoma associated RAD50 D69Y hotspot mutation disrupts DNA repair fidelity and promotes genome instability**.

II. Background and Significance

DNA double-strand breaks (DSBs) are among the most cytotoxic forms of genomic damage and arise from both endogenous sources such as replication stress and exogenous insults such as ionizing radiation or chemotherapeutic agents [1]. Eukaryotic cells have evolved multiple conserved repair pathways to address DSBs, most prominently Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) [2]. HR is largely error-free and restricted to S/G2 phases, while NHEJ is active throughout the cell cycle but more error-prone [3,4]. Other than these two conventional repair pathways there is a third more mutagenic pathway called alternative end-joining (alt-EJ), operating even when HR and NHEJ are functional [5,6]**.** This alt-EJ pathway is commonly upregulated in cancers and is most notable in HR-deficient cancers as it becomes essential for cancer cell survival [7-10].

DSB repair pathway choice is tightly regulated by a network of proteins (Fig 1), including the Mre11-Rad50-Xrs2/Nbs1 (MRX/N) complex and Ku70/80 heterodimer. The central role of MRX complex is to tether the loose DNA ends mainly through Rad50 [11,12] and to initiate resection using the nuclease activity of Mre11 [13,14]. Ku binds DNA ends and protects them from nucleolytic degradation and subsequently recruits other NHEJ factors such as Lif1-Dnl4 and Nej1, and completes repair by ligating the DNA ends[15-19]. In S/G2, Sae2 activates Mre11 to initiate 5’ resection, enabling long-range processing by Exo1 and Dna2-Sgs1 [14,20,21].

RAD50 and cancer. Rad50 is structurally complex, comprising of ATPase head domains, a central coiled-coil, and a C-terminal hook domain that mediates dimerization and end-tethering [24–26]. Mutations in components of MRX/N complex are associated to approximately 5% of tumors and out of those many mutations are associated to Rad50 [22,27]. The clinical sequencing program at Memorial Sloan Kettering Cancer Center (MSKCC) identified several cancer-associated RAD50 alleles and modeled them in budding yeast [28].

RAD50 D69Y hotspot. Aspartate-69 is highly conserved within the Walker A motif (Fig 1), essential for ATP binding/hydrolysis. The **D69Y mutation is recurrent in lung adenocarcinoma** and annotated as a mutational hotspot across cancers [22,29]. Functional modeling shows rad50-D69Y supports MRX/N complex formation and DNA binding but compromises ATP binding and ATM/Tel1 activation, a “separation-of-function” defect [27]. Mouse models confirm its biological relevance, showing partial repair defects without complete loss of MRN function [30].

Significance in lung cancer: In the context of replication stress and carcinogen exposure, RAD50 D69Y is predicted to bias repair away from high-fidelity HR toward mutagenic EJ. This shift would accelerate genomic instability, fuel tumor evolution, and alter therapy response. Despite its recurrence, the mechanistic and clinical consequences of RAD50 D69Y remain unknown.

Understanding how RAD50 D69Y alters repair mechanisms will uncover how lung cancer cells tolerate and exploit DNA damage repair defects. These insights may identify biomarkers of repair pathway dependence and therapeutic vulnerabilities.

Central Hypothesis: The RAD50-D69Y mutation biases DSB repair toward error-prone EJ at the expense of HR, driving genomic instability in lung cancer.

III. Research Plan and Methodology

Aim: Determine the molecular consequences of the rad50-D69Y mutation in DSB repair.

Approach 1: Define how RAD50 D69Y alters DSB repair pathway usage

We observed that rad50-D69Y single mutants are not sensitive to MMS, but show synthetic sensitivity when combined with *exo1*Δ (Fig 2), and similar genetic interactions in *sgs1*Δ double mutant combinations (data not shown). These interactions support the idea that rad50-D69Y impairs HR.

To probe EJ, we used a yeast strain where end-joining is the only available repair mechanism upon galactose induction. Nej1 is a canonical EJ factor, therefore NEJ1 deleted cells cannot grow on GAL. Interestingly, rad50-D69Y grew better than WT, showing a shift towards error-prone EJ pathway (Fig 2).

Repair kinetics will be quantified by qPCR at the HO cut site in rad50-D69Y mutant by utilizing the primers at the exact HO cut site to quantify the repair of the break. We will induce DSB by exposing the cells to Galactose and after DSB induction the cells will be washed and released in Glucose to enable the repair. We will take the time points and estimate the level of repair by qPCR. In a related approach, we will assess the impact of rad50-D69Y on the type of mutations and genomic rearrangements around the break site. We will also assess mutational signatures at the DSB by sequencing repair junctions. This will distinguish small indels from larger deletions, which are often hallmarks of error-prone repair mechanisms [26].

Approach 2: Determine the mechanistic defects in MRX/N function caused by RAD50 D69Y

Rad50 plays a structural role in DSB **end-tethering**. We will use an established **HO endonuclease-DSB (HO-DSB) system** where DNA regions near the break are fluorescently tagged (GFP and mCherry) to measure end-tethering efficiency via colocalization (Fig 3) [26]. Decreased colocalization in rad50-D69Y mutants would reflect compromised tethering, a known cause of genome instability.

To evaluate the extent of DSB **end-resection**, we will use a qPCR-based resection assay targeting RsaI restriction sites located 0.15 kb and 4.8 kb from the HO-DSB. Absence of PCR product indicates intact DNA (no resection); amplification implies resection has progressed beyond the RsaI cut site [23,26]. Our early data suggest reduced resection in rad50-D69Y, consistent with impaired HR usage (Fig 4). We will perform these assays in synchronized G1 or S phase cells and in *nej1*Δ double mutants to assess crosstalk between Rad50 and EJ-specific factors.

We will perform **ChIP-qPCR** using the HO-DSB system to monitor recruitment of HR proteins (Sae2, Exo1, Sgs1) and EJ proteins (Ku70, Nej1, Lif1, Dnl4). This will identify recruitment defects at the DSB in rad50-D69Y mutants and help determine whether repair defects arise from failure to recruit HR factors or inappropriate retention of EJ components.

Impact statement

Genomic instability is a major hallmark of cancer and is particularly relevant in lung adenocarcinoma, where chromosomal aberrations and structural rearrangements are frequent and often drive disease progression. Central to maintaining genome integrity is the accurate repair of DNA double-strand breaks (DSBs) which is one of the most lethal forms of genomic damage. Failures in this repair system lead to mutagenesis, loss of heterozygosity, and chromosomal translocations—events that contribute to tumor initiation, progression, and therapeutic resistance.

Rad50, as part of the MRX/N complex (Mre11-Rad50-Xrs2/Nbs1), plays a critical and evolutionarily conserved role in sensing, tethering, and initiating repair at DSB sites. Recent clinical sequencing efforts have identified recurrent mutations in RAD50 across various tumor types, including a notable hotspot at residue D69 in lung adenocarcinoma. However, the functional significance of this mutation in DSB repair and tumorigenesis remains unresolved.

This research addresses a critical gap in our understanding of how lung cancer-associated RAD50 mutation, specifically the D69Y allele, disrupt DSB repair pathway choice in favor of error-prone mechanisms. Our preliminary data in a well-established yeast model system demonstrate that the Rad50-D69Y mutation impairs DNA end resection and enhances reliance on mutagenic end-joining pathways, potentially mirroring the genomic instability observed in human lung tumors harboring this mutation. By dissecting the molecular and genetic consequences of Rad50-D69Y, we will uncover how this mutation compromises the fidelity of DSB repair, promotes mutational burden, and fuels tumor evolution.

The outcomes of this study have significant implications for lung cancer biology:

- Provide mechanistic insight into a recurrent mutation found in lung adenocarcinoma, thereby deepening our understanding of tumor etiology.
- Help identify DNA repair vulnerabilities specific to RAD50-mutant tumors, informing the design of targeted therapies or synthetic lethal strategies in precision oncology settings.
- Define biomarkers of RAD50 dysfunction in cancer.
- Lastly, the conservation of Rad50 function across species ensures that findings from this project will be directly translatable to mammalian systems and human cancer.

In summary, this project directly addresses the biological and clinical relevance of a lung cancer-associated RAD50 mutation, with the potential to guide future diagnostic, prognostic, and therapeutic strategies for patients with genomically unstable lung cancers.

Public Non-scientific Summary

Our DNA is constantly under attack from natural processes inside our cells and from external sources like radiation or chemotherapy. One of the most dangerous types of DNA damage is called a double-strand break, where both strands of the DNA molecule are cut. If this kind of damage isn't repaired properly, it can lead to serious problems such as mutations, chromosomal errors, and even cancer.

Fortunately, our cells have built-in DNA repair mechanisms that fix these breaks. One of these mechanisms is very accurate, while others are more error-prone. In cancer, the balance between these repair mechanisms is often disrupted, leading to greater instability in the genome. This instability is a major factor that allows cancer cells to grow uncontrollably.

This project focuses on a specific protein coding gene called RAD50, which helps organize and repair broken DNA. In patients with lung cancer, scientists have found a recurring mutation in RAD50. This mutation changes just one "letter" in the protein code but may have major effects on how DNA repair works.

We will study how this lung cancer-associated mutation affects DNA repair. Our early results suggest that the mutated version of RAD50 shifts DNA repair away from the accurate DNA repair mechanism toward a more error-prone one. Over time, this could lead to the kinds of genetic changes that help cancer develop or become resistant to treatment.

By understanding how this RAD50 mutation affects DNA repair, we hope to gain fundamental knowledge about etiology of lung cancer and whether RAD50 can be targeted with new or existing therapies. This research could ultimately help improve diagnosis and treatment strategies for patients whose tumours carry similar genetic defects.

Budget

Budget Summary:

1.	Research Supplies	$7,000 (1-year)
2.	Personnel (1.5 coop student researcher)	$18,000 (1-year)
	Total Requested	$25,000 (1-year)

Detailed Budget Justification:

1. Research Supplies – $7,000
This project involves significant molecular biology work using yeast as a model system to study lung cancer-associated mutation in RAD50. Key consumables and reagents include:

- Yeast media components and Molecular biology reagents: $2,000
- Chromatin Immunoprecipitation (ChIP)-qPCR kits and reagents (antibodies, magnetic beads, SYBR Green reagents): $3,000
- DNA purification kits and DNA Sanger sequencing to catalog the mutational signatures at the break site: $2,000

These supplies will allow us to carry out experiments related to DSB repair, end-tethering, resection quantification, ChIP-qPCR, and mutational analysis.

2. Personnel – $18,000
One coop studentship (100% time, 4 months) = $3000/month x 4 = $12,000
One coop studentship (50% time, 4 months) = $1500/month x 4 = $6,000
The students will be working on this project with me in the lab and under my direct supervision.

Education/experience level of students:

- Third-fourth year undergraduate student researcher in Biochemistry and Molecular biology.
- Supervised by me, with weekly meetings and continuous guidance with the aim of completing this project within the period.

Role and commitment:

- 1 student 100% and 1 student 50% devoted to this project.
- Will perform the experiments: yeast culturing, strain construction, PCR assays, DSB induction, ChIP-qPCR, fluorescence imaging, and data analysis.

APPENDIX – Figures (2 pages) and References (2 pages)

Figures

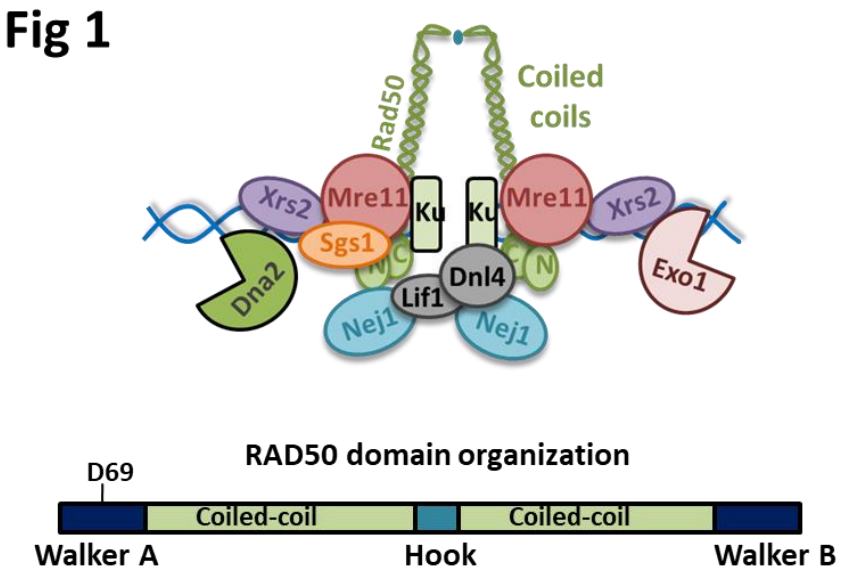

Fig 1. Schematic representation of factors at DSB (top); and Schematic representation of Rad50 domains with the D69Y lung cancer mutant (bottom).

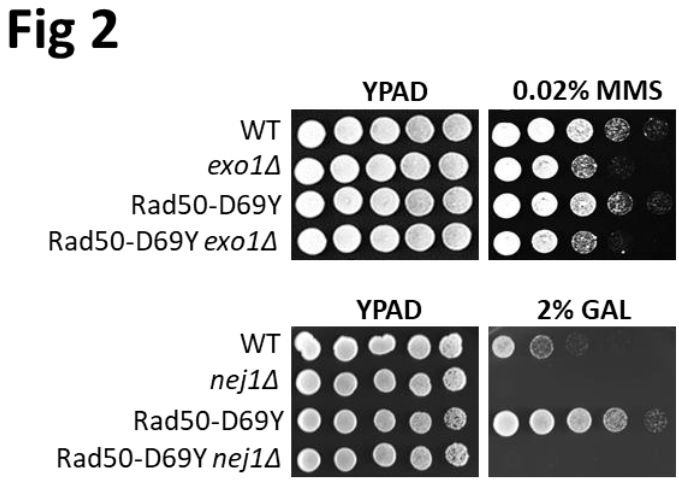

Fig 2. Drop assay of Rad50 mutants with *exo1*Δ and *nej1*Δ on MMS and Gal, respectively.

Fig 3

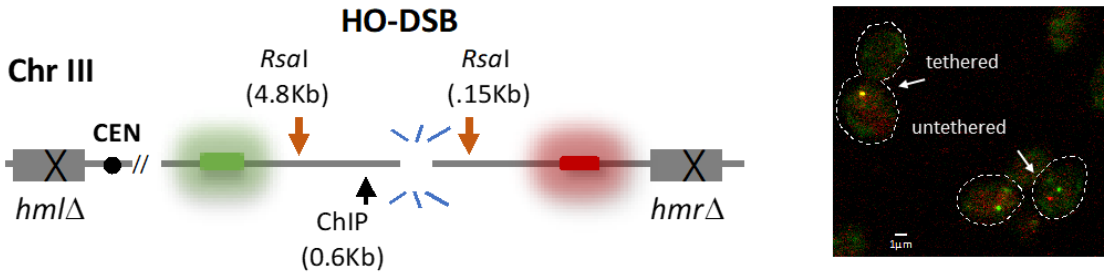

Fig 3. Schematic representation of the engineered strain with HO-DSB cut site (right); Representative image of yeast cells with tethered and untethered ends (left).

Fig 4

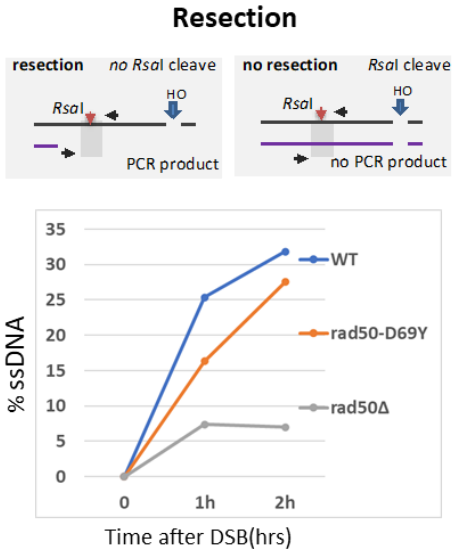

Fig 4. Schematic representation of RsaI cut site used in the qPCR resection assays. Resection of DNA at DSB site as measured by percentage single-stranded DNA (ssDNA) at 0, 1 and 2h post DSB induction in cycling cells.

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September 16 , 2025

Lung Cancer Canada
Geoffrey Ogram Memorial Research Grant Committee

RE: Institutional Support for Dr. Aditya Mojumdar

Dear Members of the Adjudication Committee,

As Chair of the Department of Biochemistry and Microbiology at the University of Victoria, I am pleased to confirm strong institutional support for the application submitted by Dr. Aditya ("Adi") Mojumdar to the Geoffrey Ogram Memorial Research Grant competition.

Dr. Mojumdar holds a tenure track Assistant Professor position in our department building a research program addressing fundamental and clinically relevant questions in genome stability. His proposal, "Characterization of lung adenocarcinoma associated mutational hotspot of RAD50 in DNA double-strand break repair," leverages his expertise in DNA repair mechanisms and the powerful yeast model system to generate mechanistic insight into a recurrent RAD50 mutation identified in lung adenocarcinoma. This work is timely, innovative, and has the potential to reveal vulnerabilities that may be exploited for targeted cancer therapies.

The University of Victoria is fully committed to supporting his research program. Our department provides Dr. Mojumdar space, access to core facilities, and the infrastructure necessary to complete the proposed studies, including advanced molecular biology, genomics, and imaging resources. Importantly, Dr. Mojumdar will have unfettered access to two well-established yeast research laboratories—the Nelson Lab and the Cobb Lab—which share open concept research space. He can readily utilize all tools, equipment, and shared expertise needed to carry out the proposed experiments effectively.

The feasibility of this project is high: Dr. Mojumdar has preliminary data supporting his hypothesis, access to the required equipment and reagents, and trained personnel in place to carry out the proposed research. The budget and scope are appropriate for the one-year funding period, and the outcomes will directly advance understanding of how RAD50 mutations contribute to genomic instability in lung cancer.

On behalf of the Department of Biochemistry and Microbiology, I am pleased to endorse this application and confirm that the proposed research can be successfully undertaken at the University of Victoria. We view this project as an important contribution to the field of cancer biology and as a valuable training opportunity for undergraduate and graduate students. Thank you for your consideration of Dr. Mojumdar's application.

Yours sincerely,

Jennifer Cobb

Jennifer Cobb, PhD
Professor and Chair, Dept. Biochemisty and Microbiology