Investigating the role of Cited-1 in promoting Wnt induced tumorigenesis in intestines

By:

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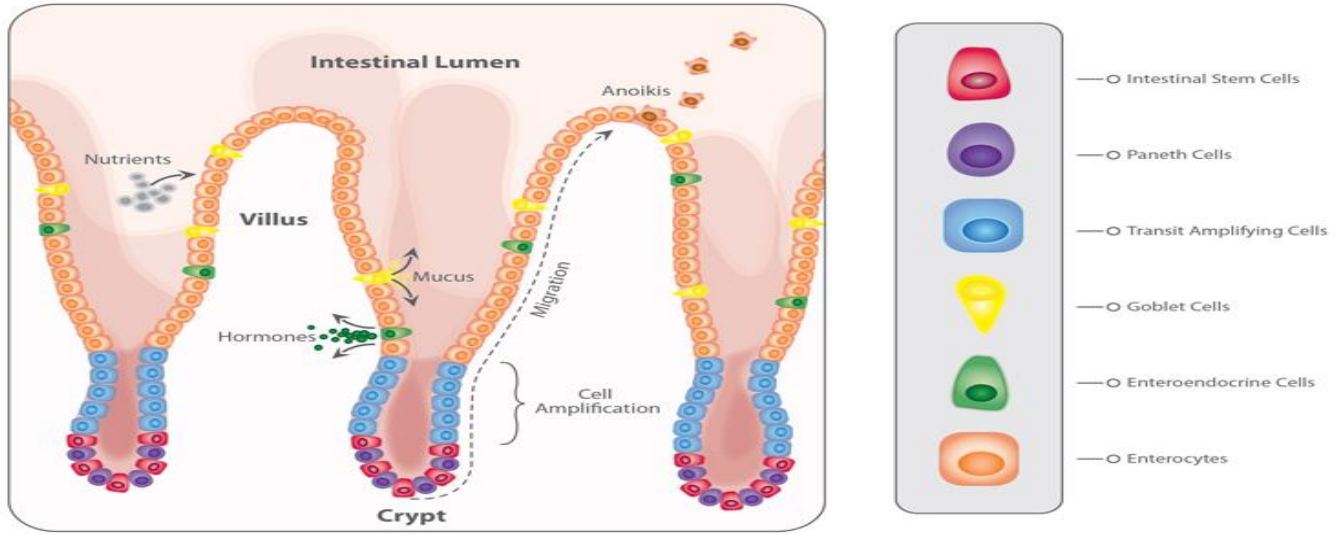
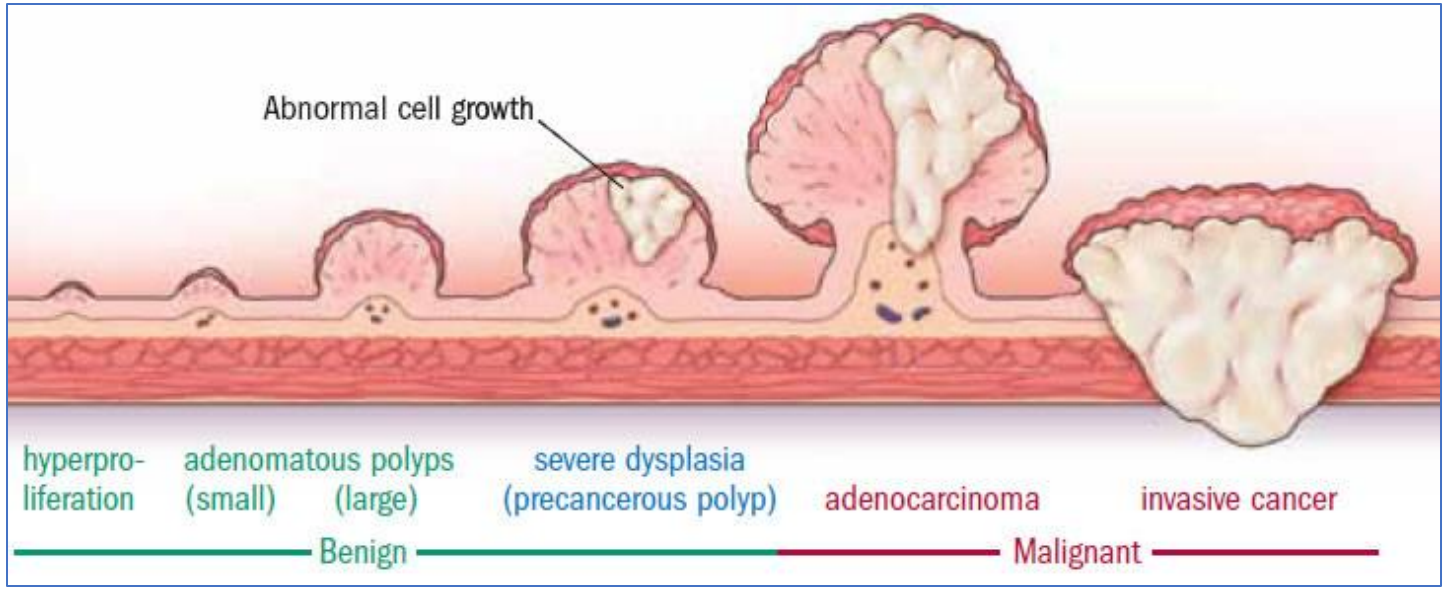
Background

- Colorectal cancer arises from epithelium of intestinal mucosa.
- Aberrant activation of *Wnt* signaling due to mutation in *Apc* (adenomatous polyposis coli) is a major cause of colorectal cancers.
- Microarray analysis of *Apc* knockout (conditional) mice model revealed in overexpression/mis-expression of certain genes like Ephb4, c-Myc, Cited-1 etc.





Source – Eisenmann D et.al., 2005, https://gottliebgi.com/colonoscopy-polypectomy-and-colon-cancer-prevention

What is already known on this subject?

- Cited-1 gene plays a key role during embryonic development, by regulating the expressing in extraembryonic tissues and in trophectoderm-derived cells of the placenta.

- Transcriptional regulation of Cited-1 gene is seen in exclusively in the nephrogenic progenitor cells. It is clearly detectable in the nuclear compartment of Wilms' tumor blastema, indicating that Cited-1 is a diagnostic marker.

- Papillary thyroid carcinoma is found to have up regulated Cited-1 expression after evaluation by tissue microarrays and immunohistochemistry.

- Cited-1 is also found as a trans activator nuclear protein and is expressed in various melanocytes, breast epithelial cells, embryonic tissues.

Aims and Objectives

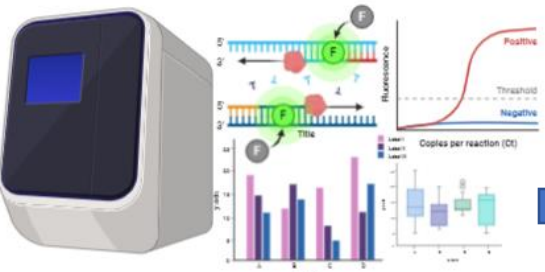
To delineate the propensity of the functional role of Cited-1 in promoting Wnt- induced tumorigenesis in intestines.

1. Determination of Cited-1 expression in colorectal cancer patient samples/cell lines of different grades compared to healthy controls.

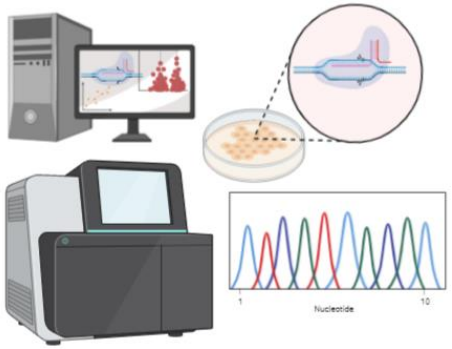
2. Generation of knock-in/out Cited-1 cancer cell models by CRISPR/Cas9 systems.

3. Deciphering the functional role of Cited-1 knock-in/out cell models in proliferation, migration, and invasion

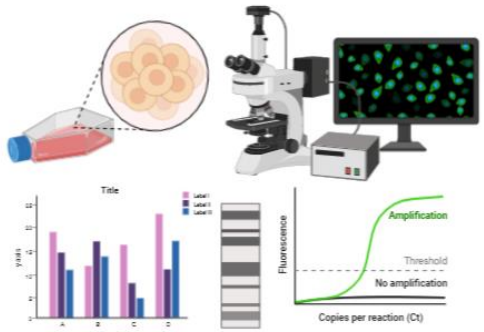
4. Development and characterization of Cited-1 overexpression transgenic murine models using i-GONAD CRISPR/ Cas9 approach



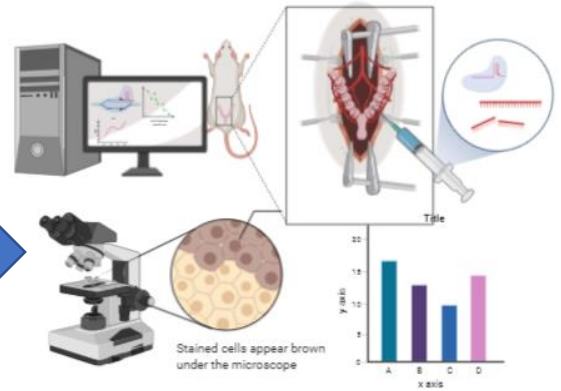
Sample collection, Total RNA isolation, TaqMan Individual assays



gRNA design, Cloning, Transfection, PCR and Sequencing



Immunocytochemistry, Invasion assay, MTT assay, Scratch assay



CRISPR/ Cas 9 reagent design, GONAD procedure, Immunohistochemistry

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Methodology/Milestones

1. Determination of Cited 1 expression in colorectal cancer patient samples/cell lines of different grades compared to healthy controls

	0th	12th	24th	36th	48th	60th
Sample collection, Reagents procurement, Optimization of RNA isolation, Total RNA isolation, cDNA synthesis						
TaqMan individual assay validation with compared to adjacent normal tissues						

Sample Collection → Total RNA isolation and enrichment → TaqMan Individual assays → Analysis and interpretation

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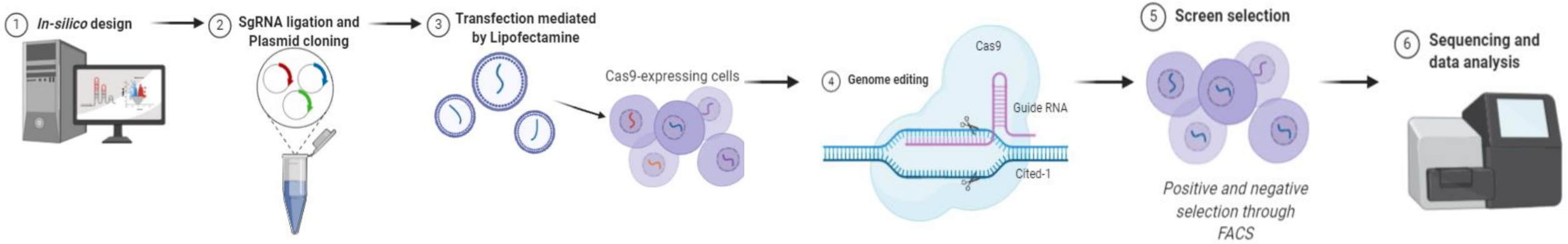
Methodology/Milestones

2. Generation of knock-in/out Cited-1 cancer cell models by CRISPR/Cas9 systems

	0th	12th	24th	36th	48th	60th
Sequence search, SgRNA design and cloning, Cas9 constructs, donor DNA design						
Vector construction and ligation, Transfection, FACS sorting, PCR and Sequencing, Establishment of new knock-in/out Cited-1 cell lines models						



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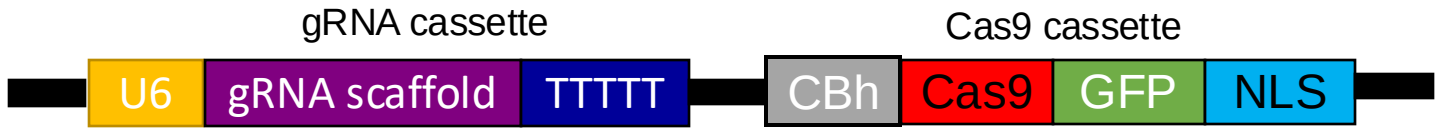
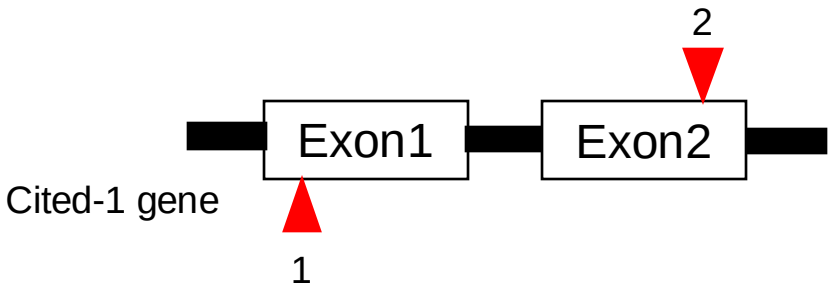
Methodology/Milestones

Vector Map (gRNA ligated with pCas9 plasmid)

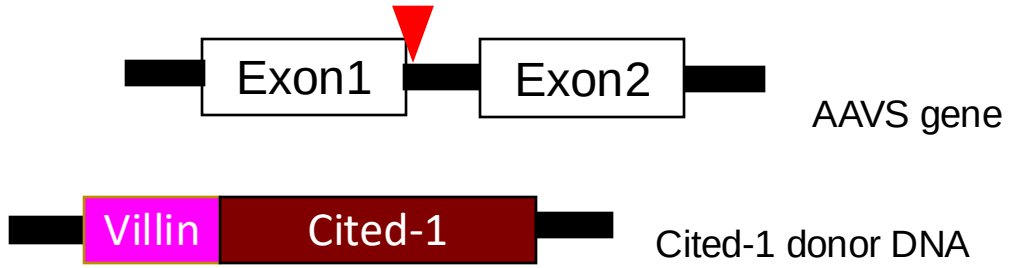


For knock out



gRNA 1-TGCCGCGCCGGCGCCCGCGA
gRNA 2-CCCGGTTCCGCGGCTCCATC

For knock in



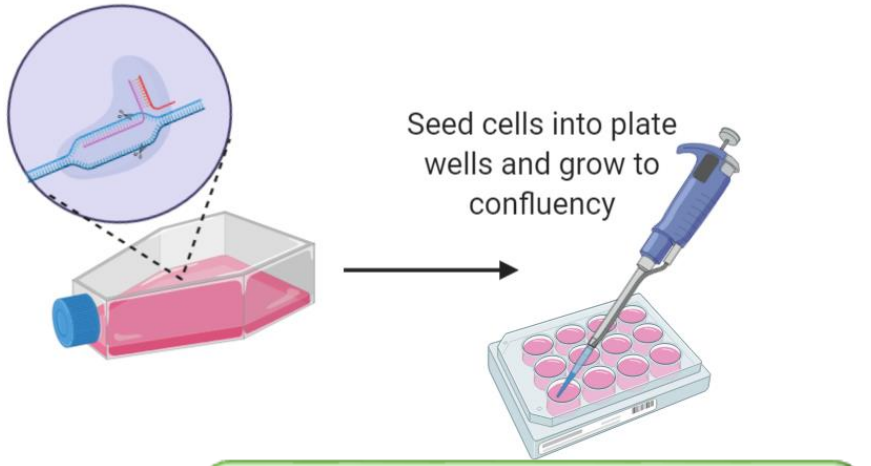
gRNA-CGACCGGTTCGCGGTAGCGG

Transfection

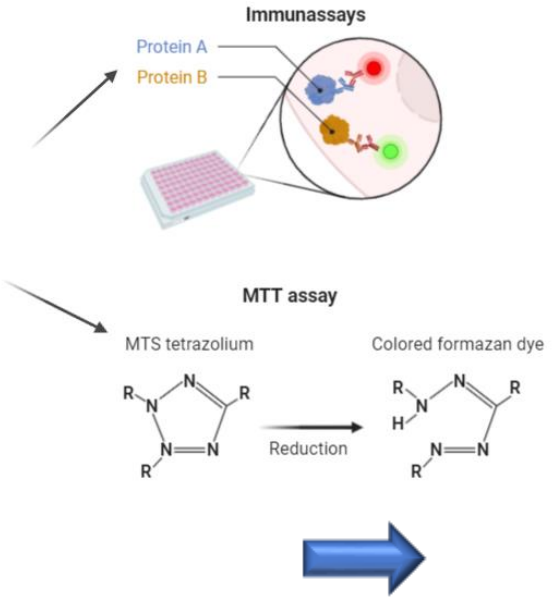
Methodology/Milestones

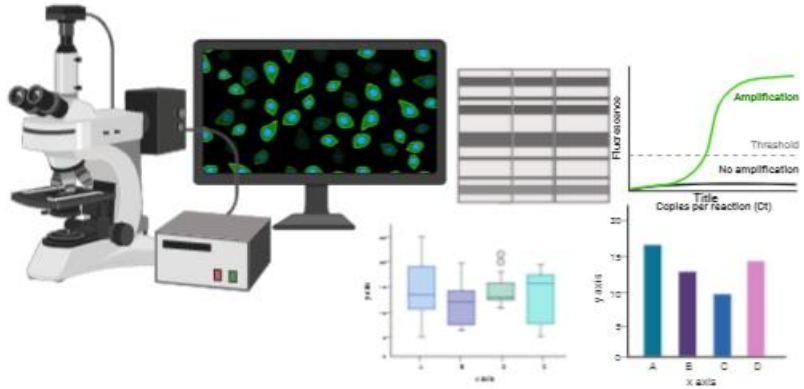
3. Deciphering the functional role of Cited 1 knock-in/out cell models in proliferation, migration, and invasion

	0th	12th	24th	36th	48th	60th
Determination of % viability by using MTT assay, Immunocytochemistry of the knock-in/ out cell models						
Determination transfection efficiency by invasion and migration by wound healing assay and Boyden chamber approach						
Analyzing the gene expression by q-PCR and immunoblots						



Cited-1 cell knock in/out models seeded, MTT assay, Wound healing and Boyden Chamber





Analyzing miRNA-gene interactions

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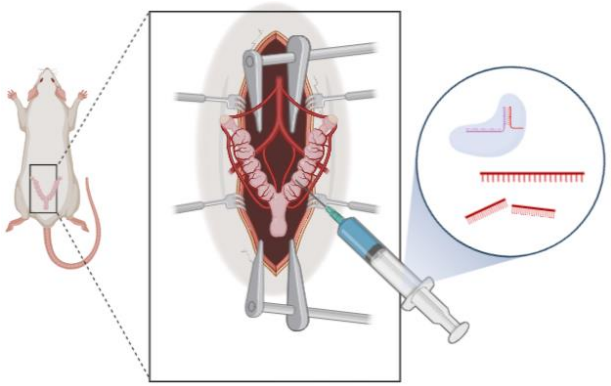
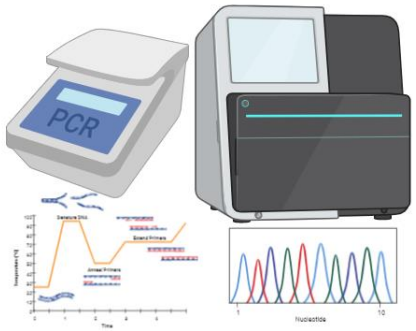
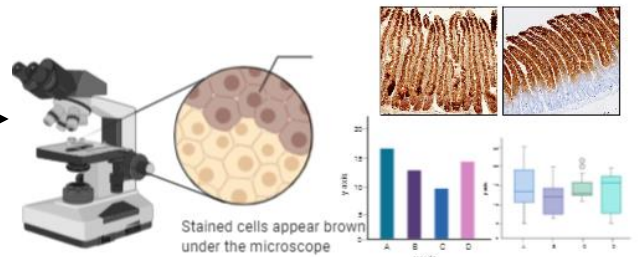
Methodology/Milestones

4. Development and characterization of Cited 1 overexpression transgenic murine models using i-GONAD CRISPR/ Cas9 approach

	0th	12th	24th	36th	48th	60th
Design of single stranded donor DNA, tracRNA and gRNA design and construction, Optimization of CRISPR/ Cas9 genome editing solutions, and Technical skill development in surgical methods				■	■	
Generation of transgenic using GONAD CRISPR/Cas9 procedure, Assessment of Knock in efficiency, Dissections, Tissue Processing, Embedding, Sectioning and Immunohistochemistry					■	■







In-silico design of sgRNA, editing solutions, donor DNA

Development of *in-vivo* models using GONAD CRISPR/ Cas9 method

Analysis of transgenic mice using PCR and sequencing

Characterization and functional evaluation

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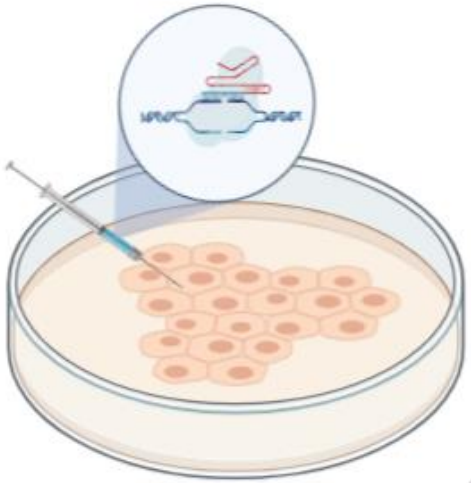
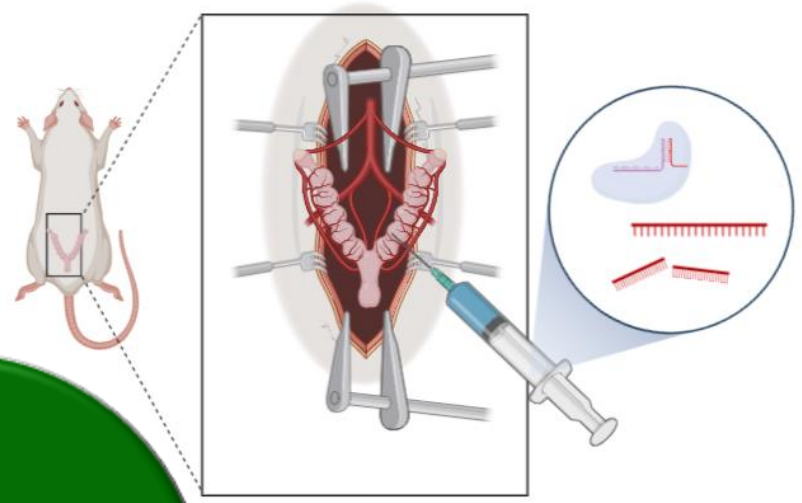
Challenges/Risk factors



Challenges

Generation of knock in/out cell models using CRISPR/ Cas9

GONAD CRISPR/ Cas9 procedure



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Deliverables/ Outcome

- Gain insights into the underlying molecular mechanism of intestinal tumorigenesis.
- Establish Cited-1 as a potent detrimental factor in colon cancer initiation leading to polyp formation.
- Cited-1 might be a differential biomarker in early colorectal cancer pathogenesis.
- Publications to bring out the utility of the study.

References

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Thank You
For Your Attention

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