Research Achievements of Prof. Ganesh Nagaraju

Germline mutations in the genes that regulate homologous recombination (HR) and genome integrity cause various genetic disorders including Fanconi anemia (FA). Mammalian RAD51, an ortholog of bacterial RecA protein plays a key role in HR and genome maintenance. Mammalian genome encodes five RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3). Germline mutations in RAD51 paralogs cause FA-like disorder and breast and ovarian cancers. Using genetic, cytogenetic and cell biological as well as biochemical approach, the nominee demonstrated that *RAD51C* (*FANCO*) is indeed a novel gene in the FA pathway of DNA interstrand cross-link (ICL) repair. Our group showed that RAD51C plays a downstream role in the FA pathway ICL repair. More interestingly, they find that RAD51C regulates intra-S-phase checkpoint and repair distinctly. These findings have implications for FA and breast and ovarian cancer susceptibility **(*JBC*, 2012)**. We identified that XRCC3 S225 is a novel phosphorylation target of ATM and ATR kinases. We also demonstrated that XRCC3 S225 phosphorylation is crucial for DNA double-strand break (DSB) repair by HR and intra-S-phase checkpoint regulation as well as maintenance of genome integrity **(*MCB*, 2013)**. These contributions were recognized with **B.M. Birla Science Prize in Biology to the nominee for the year 2012.**

Replication forks are susceptible for breakage if unprotected when the forks stall due to template damage, various secondary structures and DNA bound proteins. BRCA2 tumor suppressor and FA pathway proteins are known to protect the stalled forks. We showed that RAD51 paralogs in distinct complexes protect and restart the stalled forks. We demonstrated that RAD51 paralogs protect the stalled forks in a non-epistatic manner to BRCA2. Notably, the pathological mutants of RAD51C were defective for fork protection, implying the tumor suppressor and essential functions of RAD51 paralogs in genome maintenance **(*NAR*, 2015)**. These contributions were recognized with **National Bioscience award from DBT and Sir CV Raman young scientist award from Government of Karnataka for the year 2015.**

Our study demonstrated that FANCJ helicase suppresses gene duplication/amplifications during repair of DSBs by sister chromatid recombination, providing insights into the mechanism of pathological repair leading to tumorigenesis **(*NAR,* 2017).** Our group recently demonstrated that RAD51 paralogs localize to mitochondria, facilitate mitochondrial DNA replication and maintain its stability **(*MCB,* 2018).**

Psoralen is widely used as an anticancer agent and it requires activation by UV-A radiation which is carcinogenic in nature, and patients are susceptible for developing skin cancer. To circumvent this problem, in collaboration with Prof. A.R. Chakravarty, nominee has developed a novel ICL molecule (VDC-visible light inducible crosslinker) which can be activated by visible light. They demonstrate that photoactivation of VDC induces prolonged activation of cell cycle checkpoint and high degree of cell death in cancer cells as well as FA/HR pathway defective cells. Their results imply that VDC has the potential to be used in cancer therapy and suggest that tumors arising from the patients with gene mutations in FA and HR repair pathway can be specifically targeted by a photoactivatable VDC **(*Carcinogenesis*, 2016).**

The G-rich sequence motifs in all organisms have the potential to form G-quadruplex secondary structures. Such sequence motifs are abundantly present in gene promoters, telomeric sequences and DSB hot spots. However, the significance of G-quadruplexes and the helicases that resolve G4 structures in prokaryotes is poorly understood. *Mycobacterium tuberculosis* genome is GC rich (65%) and there are >10,000 G-rich motifs that have the potential to form G4 structures. We demonstrated that *M. tuberculosis* DinG helicase unwinds G4 structures and also provided evidence for the existence of G4 structures in the promoters of *M. tuberculosis* genes **(*JBC*, 2014).** The pathways and the mechanisms by which *M. tuberculosis*, a slow growing human pathogen responds to different types of replication stress and DNA damage is unclear. Replication fork reversal is emerging as an evolutionarily conserved physiological response for restart of stalled forks. Our work demonstrated that *M. tuberculosis* RecG helicase drives efficient reversal of stalled forks. This has implications for replication restart mechanisms in mycobacteria **(*JBC,* 2015).** Overall, for all the contributions upto 2017, prestigious **Shanti Swarup Bhatnagar prize was awarded in Biological Sciences for the year 2018.**

Oncogene activation induces replication stress by depletion of dNTPs which leads to genome instability and cause tumorigenesis. The essential functions of RAD51 paralogs in genome maintenance and tumor suppression is largely unknown. In this direction, our recent study identified a novel function of XRCC2 in regulating replication fork progression during dNTP alterations. This work provide insights into how a normal cell can become cancerous, and how XRCC2 can protect the genome from replication stress and prevent tumorigenesis (***Cell Reports*, 2018**). Our work demonstrates the repair independent functions of RAD51 paralogs in regulating replication fork progression and genome maintenance. When the stalled fork collapses, it requires participation of HR for its repair and recovery. Our recent study shows that two of the RAD51 paralogs; XRCC2 and XRCC3 are distinctly activated by ATR kinase to prevent replication stress, repair and restart the stalled/collapsed forks and maintain genome homeostasis (***Cell Reports*, 2019**).

The SCR associated gene amplifications could arise by defect in DNA end resection or due to impaired displacement of nascent strand from the D-loop structures during recombinational repair of DSBs. FANCJ being a helicase could regulate both these events. In this direction, using an ER-*Asi*SI system to score for DNA end resection, we have demonstrated a novel function of FANCJ helicase in DNA end resection. By chromatin IP studies we showed that FANCJ helicase promotes DNA end resection by recruiting CtIP to the sites of DSBs. Notably, FANCJ-CtIP mediated DNA end resection is dependent on FANCJ phosphorylation and acetylation. CDK mediated phosphorylation of FANCJ promotes FANCJ acetylation and its interaction with CtIP. FANCJ phosphorylation is also required for its interaction with BRCA1 at damaged DNA sites, thereby facilitating CtIP recruitment and DNA end resection. In addition to scaffolding role of FANCJ in CtIP recruitment, its helicase activity is also crucial for end resection. These data identify a novel function of FANCJ helicase in promoting DNA resection and provide mechanistic insights into its role in the repair DSB repair HR and genome maintenance (***PLoS Genetics*, 2020**). For 2018, 2019 Cell Reports and 2020 PLoS Genetics, the **JC Bose Fellowship was awarded in 2021**.

Awards/Honors

1. Prof. K.V. Giri memorial award for best Ph.D thesis of the Department of Biochemistry, Indian Institute of Science, Bangalore (2003)
2. Lady Tata Memorial Trust, UK, international award for Leukemia research (2009)
3. B.M. Birla Science Prize in Biology (2012)
4. National Bioscience Award for Career Development from DBT, India (2015)
5. Sir C.V. Raman Young Scientist award from Government of Karnataka (2015)
6. Secretary, Society of Biological Chemists (SBC), India (2017)
7. Fellow of the National Academy of Sciences, India (NASI) (2018)
8. Shanti Swarup Bhatnagar Prize (2018)
9. Recipient of exceptional faculty funds from DBT-IISc partnership programme (2019)
10. Sreenivasaya Memorial Award from SBC(I) (2020)
11. Fellow of the Indian National Science Academy (INSA) (2021)
12. Sir J.C. Bose Fellowship (2021)
13. Fellow of the Indian Academy of Sciences (FASc) (2023)
14. Har Gobind Khorana Endowment Lecture (2023)