Ten Best Papers of Prof. Ganesh Nagaraju with Highlights of Contribution

1. Dixit, S., Bhattacharya, D., Saxena, S., Sahoo, S., Nagraj, T., Chittela, R., Somyajit, K., and **Nagaraju, G.** (2024). RTEL1 helicase counteracts excessive homologous recombination to safeguard replicating genomes. ***Cell Reports*** **43:114594.**

   Homologous recombination (HR) is an important pathway for the repair of DNA double-strand breaks, genome maintenance and tumor suppression. However, unregulated HR can lead to chromosomal rearrangements and genome instability. This work demonstrates the role of RTEL1 helicase in suppressing hyper-recombination during DNA replication and facilitating error-free genome duplication. The hyper-recombination and replication defects in RTEL1-depleted cells can be rescued by co-depletion of RAD51 and RAD51 paralogs. RTEL1 interaction with PCNA and its helicase activity is required for suppressing HR during DNA replication. Our data identify a novel role of RTEL1 helicase in restricting RAD51-mediated HR and fork reversal to facilitate error-free genome duplication.

2. Nath, S., and **Nagaraju, G.** (2020). FANCJ helicase promotes DNA end resection by facilitating CtIP recruitment to DNA double-strand breaks. ***PLoS Genetics*** **16(4): e1008701.**

   Homologous recombination (HR) has been considered as an error-free pathway in repairing DSBs and maintaining genome stability. Cyclin-dependent kinases (CDKs) and various factors, including MRE11, CtIP, EXO1, and BLM helicase, participate in DNA end resection to promote HR-mediated DSB repair. Despite the identification of FANCJ helicase's role in HR and tumor suppression, the molecular mechanism by which FANCJ helicase participates in HR is obscure. Here, we show that FANCJ helicase controls DNA end resection by recruiting CtIP to the sites of DSBs. The loading of CtIP is dependent on FANCJ acetylation which is mediated by CDK dependent phosphorylation of FANCJ. Moreover, in addition to FANCJ-mediated CtIP recruitment, its helicase activity is also essential for DNA end resection. Our data identify FANCJ as a novel player in the DNA end resection and provide insights into its role in HR-mediated DSB repair.

3. Saxena, S., Somyajit. K, and **Nagaraju, G.** (2019). ATR signaling uncouples the role of RAD51 paralogs in Homologous Recombination and replication stress response. ***Cell Reports*** **29: 551-559.**

   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). Our work demonstrated how differential activation of XRCC2 and XRCC3 by ATR kinase safeguards genome integrity and prevents tumorigenesis. Our work also provides implications for targeting RAD51 paralogs for the treatment of cancer.

4. Saxena, S., Somyajit, K., and **Nagaraju, G.** (2018). XRCC2 regulates replication fork progression during dNTP alterations. ***Cell Reports*** **25:3273-3282.**

Coordination between dNTP metabolism and DNA replication is essential to limit replication-associated mutations and tumorigenesis. Our group has uncovered a novel role of RAD51 paralogs subcomplex (RAD51D-XRCC2) in regulating replication fork progression during dNTP alterations. Mechanistically this function is regulated by ATR signaling by phosphorylation of XRCC2. These findings have direct relevance to how mutations in RAD51 paralogs can lead to genetic disease as well as breast and ovarian cancers.

5. Mishra, A., Saxena, S., Kaushal, A., and **Nagaraju, G.** (2018). RAD51C/XRCC3 facilitates mitochondrial DNA replication and maintains integrity of the mitochondrial genome. ***Mol. Cell. Biol.*** **38:1-18.**

Germline mutations in RAD51 paralogs cause FA-like disorder and breast and ovarian cancers. Mutations in the mitochondrial genome and its instability are linked to various genetic diseases, premature ageing and cancer. The factors and the mechanism by which integrity of the mitochondrial genome is maintained are poorly understood. Our group recently demonstrated that RAD51 paralogs localizes to mitochondria, facilitate mitochondrial DNA replication and maintain its stability.

6. Nath, S., Somyajit, K., Mishra, A., Scully, R., and **Nagaraju, G.** (2017). FANCJ helicase controls the balance between short- and long-tract gene conversions between sister chromatids. ***Nucleic Acids Res.*** **45:8886-00.**

Gene amplification is commonly found in various types of cancer and the mechanism underlying such amplifications/duplications is largely unclear. Moreover, the role of FANCJ helicase in the FA pathway of ICL/DSB repair is less understood. Our recent 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.

7. Somyajit, K., Saxena, S., Babu, S., Mishra, S., and **Nagaraju, G.** (2015). Mammalian RAD51 paralogs protect nascent DNA at stalled forks and mediate replication restart. ***Nucleic Acids Res.*** **43:9835-55.**

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. Our study shows 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. The restart of the stalled forks is dependent on ATP hydrolysis by CX3 complex. Notably, the pathological mutants of RAD51C were defective for fork

protection, implying the tumor suppressor and essential functions of RAD51 paralogs in genome maintenance.

8. Somyajit, K., Mishra, A., Jameei, A., and **Nagaraju, G.** (2015). Enhanced non-homologous end joining contributes toward synthetic lethality of pathological RAD51C mutants with poly (ADP-ribose) polymerase (PARP). ***Carcinogenesis* 36:13-24.**

   BRCA1 and BRCA2 tumor suppressors are required for maintenance of genome stability via DNA damage repair by HR. Germline mutations in *BRCA1* and *BRCA2* leads to hereditary breast and ovarian cancers. BRCA1/BRCA2 deficient tumor cells can be targeted by using PARP inhibitors in a "synthetic lethal" approach. However, RAD51C pathological mutants that were identified in breast and ovarian cancer patients are hypomorphic, and targeting such tumor cells with PARP inhibitors is highly challenging. In this work we demonstrate that hypomorphic RAD51C mutant tumor cells can be targeted by enhancing the NHEJ with low dose of IR and low dose of PARP inhibitor. Our results show that cancer cells arising due to hypomorphic mutations in RAD51C can be specifically targeted by a 'synergistic approach' and imply that this strategy can be potentially applied to cancers with hypomorphic mutations in other HR pathway genes.

9. Somyajit, K., Basavaraju, S., Scully, R. and **Nagaraju, G.** (2013). ATM- and ATR-mediated phosphorylation of XRCC3 regulates DNA double strand break-induced checkpoint activation and repair. ***Mol. Cell. Biol.* 33:1830-44.**

   We identified that XRCC3 S225 is a novel phosphorylation target of ATM and ATR kinases. We 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.

10. Somyajit, K., Subramanya, S., and **Nagaraju, G.** (2012). Distinct roles of FANCO/RAD51C in DNA damage signaling and repair: Implications for Fanconi anemia and breast cancer susceptibility. ***J. Biol. Chem.* 287: 3366-3380.**

    Germline mutations in *RAD51C* and *XRCC2* are known to cause FA-like disorder. Using genetic, cytogenetic and cell biological as well as biochemical approach, the we 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.