Signed details of the excellence in research work for which the Sun Pharma Research Award is claimed, including references and illustrations.

The Sun Pharma Research award is being claimed for the following two publications:

Publication #1:

Tripathi V, Agarwal H, Priya S, Batra H, Modi P, Pandey M, Saha D, Raghavan SC, **Sengupta S** (2018). MRN complex-dependent recruitment of ubiquitylated BLM helicase to DSBs negatively regulates DNA repair pathways. Nat Commun. 9(1):1016. PMID: 29523790.

This work from Dr. Sengupta's laboratory published in *Nature Communications* has demonstrated that how proteins maintain the integrity of the human genome during multiple steps in the DNA damage recognition and repair pathways. In this publication the authors have used a unique system by which they had introduced damage at specific locations in the human genome. This experimental design has allowed them to determine how genome stabilizers localizes to the damage sites, the kinetics of accumulation at these sites, whether the recruitment depends on which other cellular factors and finally delineates how the cell decides which repair pathway it should use to eliminate the lesion. Hence this work gives a molecular frame work to design therapeutic interventions to artificially accelerate the process by which the integrity of the genome can be sustained. A schematic diagram which summarizes the finding in this publication is presented in Figure 1.

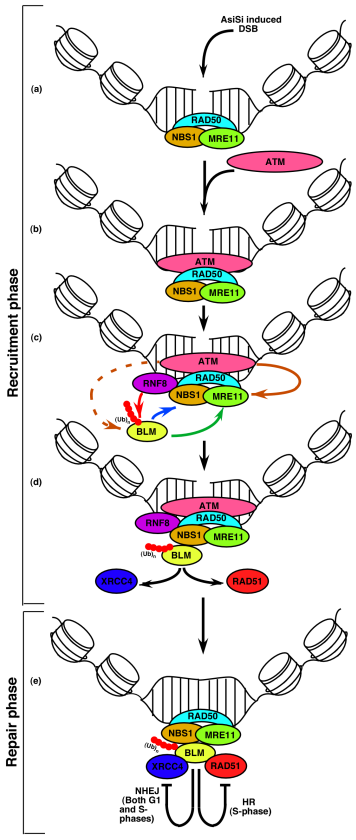

Figure 1: Mechanism of BLM recruitment to the DSBs and its effect on DNA repair. In the recruitment phase, MRN complex is recruited to the AsiSI induced DSBs (section a), which in turn recruit ATM (section b). Chromatin bound E3 ligase RNF8 polyubiquitylates BLM (red line, red dots representing ubiquitin residues). The kinase activity of ATM (brown lines) is also essential for the BLM recruitment process, acting directly on BLM or via NBS1/MRE11. Single stranded region obtained due to the exonuclease activity of MRE11 (green line) is probably an essential requirement for BLM recruitment. Additionally, polyubiquitylated BLM interact with NBS1 (blue line), which allows BLM to indirectly yet functionally interact with MRE11, allowing the optimal recruitment of BLM (section c). The helicase activity of BLM is essential for the co-

recruitment of c-NHEJ protein XRCC4 in G1 phase and RAD51 in S-phase (black lines) (section d). During repair phase, BLM predominantly inhibits c-NHEJ in G1 and HR in S-phase. As a backup regulatory mechanism, BLM also inhibits c-NHEJ in S-phase (section e). Hence BLM switches from a pro-repair protein (due to its role in the recruitment phase) to an anti-repair regulatory protein (due to its role in the repair phase), thereby maintaining genomic stability. Dotted lines represent putative mechanism while solid lines represent data from either published literature or from the present report.

Publication #2:

Hussain M, Mohammed A, Saifi S, Khan A, Kaur E, Priya S, Agarwal H, **Sengupta S** (2021). MITOL-dependent ubiquitylation negatively regulates the entry of PolgA into mitochondria. PLoS Biol. 19(3): e3001139. PMID: 33657094

This publication was a paradigm shifting work which indicated that inspite of the presence of Mitochondrial Localization Signal (MLS), a key protein for mitochondrial replication, Polymerase gamma A (PolgA), can enter into mitochondria only if it non-ubiquitylated. The work has pathological significance as PolgA mutants in progressive external ophthalmoplegia (PEO) are hyper-ubiquitylated and hence cannot enter mitochondria as they form aggregates on the outer surface of the organelle. The work also identifies and validates two processes, involving genomic manipulations, which can reactivate PolgA mutants and thereby make them re-enter the mitochondria. A schematic diagram is presented in Figure 2.

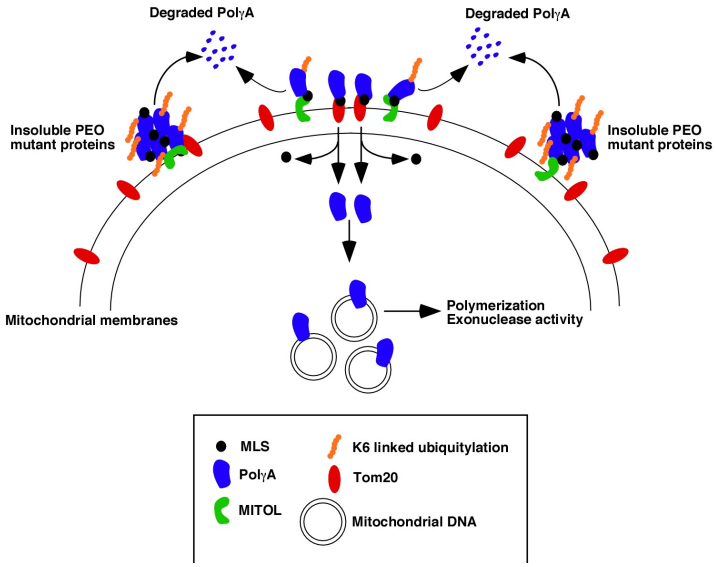

Figure 2: PolgA has a key role in mitochondrial DNA replication. Wildtype PolgA is targeted by MITOL, an E3 ligase present in the outer mitochondrial membrane. MITOL ubiquitylates PolgA at K1060 via K6-linkage. This ubiquitylation event prevents PolgA from efficiently binding to Tom20. Consequently, only non-ubiquitylated PolgA (which binds with Tom20) could enter the mitochondrial matrix, thereby allowing it to do its functions during mtDNA replication. This regulated entry of PolgA into mitochondria is no longer operational under pathological situations. In cells expressing certain PolgA variants present in PEO patients, the mutant PolgA proteins are hyper-ubiquitylated by MITOL and hence cannot enter into mitochondria. Instead these proteins are present in the insoluble fraction, which allows them to be recognized and targeted for degradation. Consequently, the lack of entry of the mutated PolgA proteins into mitochondria leads to decreased mitochondrial DNA replication and thereby diminished ability to maintain the mitochondrial genome integrity.

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