Dr.Shilpee Dutt

Inhibition of SETMAR-H3K36me2-NHEJ repair axis in residual disease cells prevent glioblastoma

Recently Dr. Shilpee Dutt's lab at ACTREC published important manuscripts in 'Neuro-Oncology' that address the fundamental issue of radiation resistance and recurrence in glioblastoma multiforme (GBM). GBM is the most aggressive and malignant primary brain tumor with median overall survival of barely 18 months. Despite multimodal therapy recurrence in GBM is inevitable making GBM the most difficult brain tumor to treat. GBM recurrence is due to the residual disease cells that are left after surgery and radio-therapy. Therefore, it is imperative to eliminate residual cells to prevent recurrence and improve patient outcome. However, because brain tumor biopsies are not available after therapy there is little understanding of the survival mechanisms of these residual cells. The only possibility to access and study these cells is by an innovative approach that models the clinical scenario of therapy resistance — an approach that is crucial for the development of effective therapies. With this aim, Shilpee Lab at ACTREC have developed a cellular model system from clinically relevant naïve primary GBM patient samples and cell lines that mimic clinical scenario of GBM resistance and recurrence. Using this system, they are able to capture the rare population of cells (residual resistant cells) that survive radiotherapy and are responsible for relapse.

In the study by Kaur et al. published in Neuro-Oncology, Dr. Dutt's group showed that irradiation induce double strand breaks (DSBs) in residual cells followed by recruitment of DSB repair proteins like ATM, ATR, Chk1 and Chk2. DSBs in a cell are repaired either by non homologous end joining (NHEJ) pathway or homologous repair (HR) pathway, Kaur et al. showed that in residual cells following radiation exposure NHEJ proteins Ku80 and Artemis are stabilized while HR proteins BRCA1 and Mre11 are downregulated. Furthermore, residual cells upregulated SETMAR, a methyltransferase and thus the corresponding histone modification (H3K36me2) levels. H3K36me2 directly recruited Ku80 to

preferentially activate NHEJ pathway. Inducible knockdown of SETMAR in residual cells significantly reduced H3K36me2, Ku80 recruitment and DDR, enforcing therapy induced irreversible senescence in these cells. Contrarily mutating H3K36A resulted in loss of Ku80 and BRCA1 protein recruitment at DSBs leading to complete apoptosis and abrogation of tumorigenicity *in vitro* and *in vivo* in orthotopic xenograft mouse model. Pharmacological inhibition of NHEJ pathway phenocopied H3K36 mutation effect, demonstrating the dependency of residual cells on NHEJ pathway. These data provide important molecular insights into the survival mechanisms of inaccessible from patient biopsies residual cells and offer therapeutic opportunity to prevent GBM relapse through eradication of residual cells by abrogating residual cell specific SETMAR- NHEJ axis.

Together, these studies shed light on the novel molecular mechanism of DNA repair adopted by clinically relevant therapy resistant residual GBM cells for their survival. By identifying druggable targets specific to the resistant residual cells, these studies have opened a new much needed window of opportunity for therapeutic intervention in Glioblastoma.

Inhibition of SETMAR-H3K36ME2-NHEJ repair axis in residual disease cells prevent glioblastoma recurrence. Kaur E, Nair J, Ghorai A, Mishra SV, Achareker A, Ketkar M, Sarkar D, Salunkhe S, Rajendra J, Gardi N, Desai S, Iyer P, Thorat R, Dutt A, Moiyadi A, Shilpee Dutt*. Neuro Oncol. 2020 May 27:noaa128. doi: 10.1093/neuonc/noaa128.