Indian Institute of Technology Delhi

School of Interdisciplinary Research (SIRe)

SIRe Project Number
2022-01-40
(will be assigned by SIRe)

PhD Project

Project Details	
Project Title	Isolation and characterization of cancer stem cells from different niches in glioblastoma
Project Summary	Glioblastoma (GBM) is the most prevalent brain cancer with limited therapeutic success. One of the primary reasons for the same is the existence of self-renewing, quiescent GBM cancer stem cells (GSCs). Recent studies have shown that GSCs are not independent entities but are in constant communication with their respective niches. GSC plasticity is determined by environmental cues, specifically, tumor vasculature, which is a key player in shaping such niches (e.g., perivascular or hypoxic niches). It has become increasingly evident that there are dynamic changes that occur within the GBM tumor governed by the local tumor tissue environment. The cellular hierarchical model of cancer stemness has been challenged and more and more studies are pointing at the existence of plasticity in GSCs that are dependent on cues for various niches that exist within the tumor. One such critical extrinsic factor that governs intra-tumor heterogeneity (ITH) and is responsible for various niches is tumor vasculature. Uneven distribution of tumor blood vessels (TBVs) results in perivascular and hypoxic niches. These specific niches, in turn, regulate glioma initiation, evolution, and resistance to therapy. This project aims to identify GSCs that exist in hypoxic niches and understand what are the cellular and metabolic reprogramming that GSCs undergo to survive and evade therapy. We further want to investigate if these properties are genetically hard-wired or malleable, and what specific receptors or ion channels they express to overcome the harsh TME. Candidate gene(s) will further be functionally validated using CRISPR/Cas9 knockout approach. GSCs in different niches will be compared to see 'niche-specific signatures' that may help in designing better therapy against GBM. ***Interdisciplinary Nature of the Project:*** **SK lab at IITD**: We have developed a unique methodology to isolate cancer cells solely based on their distance from blood vessels in intracranially implanted human glioblastoma tumors. This method referred to as 'Perfusion based Fluorescent Dye Labelling of Cells' (PFDLC) is amenable to all the high-throughput -omics (e.g., transcriptomics, metabolomics, etc) as well as functional assays (Kumar S et al, Cell Metabolism, 2019, Kumar S et al, Bio-Protocol, 2020) (Fig 1). We have already established that the differential vascular inputs creates metabolic zonation and causes intra-tumoral differences in GBM. More importantly, we have also designed a marker-independent strategy to identify GSCs exploiting their 'quiescence' property localized in the perivascular niche (Kumar S et al, Angiogenesis, 2022) (Fig 2). Experiments have validated the clustering of tumor-initiating cells around blood vessels and superior migratory/invasive capabilities of this tumor sub-population. Further experiments will use already acquired RNAseq and metabolomics databases of genes and products thereof selectively enriched in tumor cells adjacent to vessels in an attempt to uncover microenvironmental cues conducive for the acquisition of these phenotypes. **SM lab at AIIMS:** In our 'Stem Cell Facility lab' here at AIIMS, we have trained staff for working with cancer and cancer stem cells. Another advantage which we have is the procurement of GBM patient samples due to the ease in access to the patient samples and established collaborators from neurosurgery department. We also have prior

	expertise of working with glioma and hypoxia as detailed below. Our lab is also well equipped with facilities to mimic hypoxic conditions (hypoxia chamber/incubator). We have studied how hypoxia influence on stemness and differentiation of dental pulp derived stem cells (DPSCs). We also have a CO2 incubator for setting of hypoxic conditions in vitro and have published few articles as well. We studied the impact of hypoxia on DPSCs in vitro and observed that hypoxia promoted stemness through decreasing cell division rate and reduced differentiation. Using glioma as a model in another independent study, we demonstrated that an active demethylation in hypoxia by TET1 and 3 as a mechanism of Oct4 and Nanog overexpression thus contributing to the formation of CSCs (Prasad P et al, 2017). We also have a well-established animal house facility in AIIMS. Our lab also has a stereotaxic instrument (Singh M et al 2021), which will be utilized in the proposed objective completion. We also have an IVIS instrument, through which non-invasive monitoring of tumor progression in vivo can be followed up.

PhD Supervisors

Role	Faculty	Academic Unit in IITD	Email ID
Supervisor 1	Saran Kumar (PI)	Kusuma School of Biological Sciences, IITD	ksaran@iitd.ac.in
Supervisor 2	Sujata Mohanty (Co-PI)	AIIMS Delhi	drmohantysujata@gmail.com

Project requirements (Student qualifications, experience required, etc)

- Masters in life sciences or equivalent degree
- DST, DBT, INSPIRE, ICMR, CSIR, GATE or other equivalent PhD fellowship qualified
- Expertise in mice handling, in vitro cell culture and in vivo studies.

Source of funding (IRD/FITT Project details, if any)

New Faculty Grant from IITD to SK
Grants from ICMR/DBT to SM

Role of Faculty Members involved:

SK (PI): Provide intellectual inputs, guidance in designing specific model system to study impact of vasculature in HCC using specific tools at my disposal and resources for performing vascular biology, tumor metabolism studies and mentoring the PhD candidate.

SM (Co-PI): Providing resources including animal facility access, intracranial implantation platform for mouse surgery, in vivo imaging, GBM patient samples, sharing resources, intellectual inputs and expertise on GBM and stem cell culture model, co-guiding PhD student.