CONCLUSIONS
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Gliomas are most common primary central nervous system (CNS) tumors in humans. In contrast to the long-standing and well-defined histopathology, the underlying molecular and genetic basis for gliomas is unclear. Many genetic alterations have been identified in human gliomas however, establishing unequivocal correlation between these genetic alterations and gliomagenesis requires accurate models for this disease. Cancer stem cells (CSC) within malignant tumors of brain, further contribute to glioma heterogeneity. In an attempt to acquire insight into the pathways responsible for malignant transformation in gliomas we began by generating several long-term glioma cultures from surgical tumor tissue samples of various grades of tumor. In this thesis, we have addressed issues related to activation of signal transduction pathways, disruption of cell cycle arrest pathways, cell-of-origin of gliomas, and possible therapeutic strategies targeting cancer stem cells.

The data presented in this thesis elaborates on:

- Development of 26 long term cultures from various grades of gliomas with six each from grade I and II, three from grade III and eleven from grade IV. The cultures are proliferative, with few showing presence of stemness markers - CD133, nestin, musashi, Sox2 and nucleostemin and cells possessed ability for multi-lineage differentiation (GFAP, Tuj1, Map2 and oligodendrocytes). Several cultures displayed ability for Neurospheres generation wherein both types - floating and semi-adherent spheres were formed by the putative glioma stem cells present in glioma tumor tissues.

- Identified human CD133-positive neural stem cells (NSC) from adult glioma tissue and established long-term in vitro culture human neuroglial culture (HNGC)-1. Replicative senescence in HNGC-1 led to
a high level of genomic instability and emergence of a spontaneously immortalized clone that developed into cell line HNGC-2 with features of cancer stem cells (CSCs). The HNGC-2 cells showed ability for self-renewal and capacity to form CD133-positive neurospheres and develop intracranial tumors supporting the cancer stem cell hypothesis.

The study highlights the importance of DNA damage elements and checkpoint kinases as key signal transducers within the complex network of genome integrity checkpoints in tumors. The study specifies an important role for genomic instability in initiation of transformed state as well as its progression into highly tumorigenic CSCs. The study signifies an important role for DNA damage elements and checkpoint kinases as key signal transducers within the complex network of genome integrity checkpoints in tumors. Loss of p53, absence of p21 and activation of ATM was found responsible for abrogated G1 checkpoint control and genomic instability in HNGC-2 cells. An aberrant expression of DNA check point control and DNA repair proteins was due to high level of replicative genomic stress leading to genomic instability. These findings suggest that this model comprised of HNGC-1 and HNGC-2 cells would be a useful system for studying pathways involved in self-renewal of stem cells and their transformation to cancer stem cells.

We hypothesize DNA damage response is responsible for immortalization of the non-tumorigenic stem cells (HNGC-1) to cancer stem cells (HNGC-2).

The activated forms of Notch and Hes isoforms were expressed in both non-neoplastic neural stem cells and brain tumor stem cells derived from it. However, they were found to be significantly overexpressed in
the brain tumor stem cells. Activation of Wnt/β-catenin canonical Wnt signaling was prominently demonstrated in in higher grades of gliomas with Wnt1 and Wnt3a being predominantly expressed. Glioma cell-lines like LN-229 and HNGC-2 cells showed high wnt activation with nuclear β-catenin accumulation. Wnt modulators/inhibitors, sFRP-1 and Dkk-1 were found over expressed in HNGC-1 cells and down regulated in cancer stem cells - HNGC-2.

✓ Suppression of wnt signaling with wnt modulator sFRP-1 over-expression in HNGC-2 cells resulted in transient down-regulation of wnt signaling resulting in lowering in β-catenin levels and concomitant inhibition of cell proliferation.

✓ Down-regulation of Wnt1 and Wnt3a in HNGC-2 and LN229 cells by their respective shRNAs resulted in reduction of nuclear β-catenin levels, cellular proliferation and in vitro migration. Wnt3a knockdown in HNGC-2 cells resulted in complete tumor regression and disease free survival in an orthotopic mouse model.

We propose that development of strategies targeting Wnt signalling pathway either by use of small molecule inhibitors or RNAi would be a powerful therapeutic tool in management of chemo-resistant Gliomas arising from cancer stem cells.