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BACKGROUND: Glioblastoma multiforme are refractory diseases and it is necessary to develop new therapies. Ion channels are membrane proteins that permeate ions and are involved in proliferation, metastasis and invasion in cancer. To date, functional analysis of ion channels in glioma cancer stem cells (GSCs) has not been done much. However, there is limited evidence regarding the ion channels in glioma cancer stem cells. **PURPOSE:** Ion channels in GSCs are identified and the anti-tumor effect by inhibitors against them is verified. **METHOD:** Cancer stem cell lines were established from patients with Glioblastoma multiforme. The whole current of cancer stem cells was measured using patch clamp technique, and electrophysiological properties and pharmacological properties were analyzed. We analyzed the effects of ion channel inhibitors on proliferation, cell death and invasion of cancer stem cells. **RESULTS:** Nonselective cationic current was observed in GSCs. This current was blocked in a concentration dependent manner by lanthanum which is a trivalent cation. Lanthanum inhibited the growth and invasion of GSCs. **CONCLUSION:** It is suggested that lanthanum inhibits cell proliferation and invasion by blocking nonselective cation channel current of GSCs.

STEM-24. CORE CLOCK GENES REGULATE CANCER STEM CELLS IN BRAIN TUMOR

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Circadian rhythms are self-sustainable endogenous 24 hours oscillations, present in every living system and cell, driving daily cycles of biological process, including cell division and metabolism. Circadian rhythm disruption by shift work or core clock genes dysfunction is associated with high risk of tumor development, indicating a putative tumor-suppressive role of circadian rhythm. Using a subpopulation of stem-like cells, coming from human glioblastoma displaying cellular hierarchies, we characterized the role of core clock genes in these stem-like cells. We found that circadian rhythm transcriptional factors Clock and Bmal1 in glioblastoma stem-like cells (GSC) is required for the growth of GSC in vitro and in vivo. Disruption of canonical circadian pathway components produce anti-brain tumor effects, including decreased proliferation, impaired self-renewal, increased cell apoptosis and depletion of GSC. Loss of Bmal1 and Clock led to mitochondrial respiration dysregulation and impaired the tricarboxylic acid cycle by regulating genes and enzymes expression in this process. Our finding established a novel role for the core circadian rhythm genes in glioblastoma stem-like cells.

STEM-25. QUAKING (QKI) LOSS ENHANCES GSC STEMNESS AND PROMOTES GLIOMAGENESIS THROUGH REGULATING INTRACELLULAR VESICLE TRAFFICKING

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Glioma stem cells (GSCs) have higher self-renewal capacity than neural stem cells (NSCs), yet the underlying molecular mechanism is less clear. Here we report the generation of a novel conditional KO allele of *Quaking (Qki)*, a tumor suppressor in glioblastoma with RNA binding activity. When *Qki* was deleted in *Nestin-CreERT2;Qki^{fl/fl}* mice, the NSC population, which is characterized by long-term BrdU retention and GFAP+/Nestin+ double positive staining, was greatly increased, indicating that *Qki* deletion enhances NSC self-renewal. To determine whether *Qki* deletion promotes gliomagenesis, we generated a *Nestin-CreERT2;Qki^{fl/fl};Pten^{fl/fl};p53^{fl/fl}* cohort, in which 92% of the mice developed glioblastoma with a median survival of 105 days. However, the *Nestin-CreERT2;Pten^{fl/fl};p53^{fl/fl}* cohort did not develop any glioma up to a year; therefore *Qki* deletion greatly promotes gliomagenesis. Transcriptomic and proteomic profiling coupled with PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation) analyses revealed that genes involved in intracellular vesicle trafficking were greatly enriched by *Qki* deletion. Specifically, 34% of the genes up-regulated by *Qki* deletion are involved in receptor delivery, and 39% of the genes down-regulated by *Qki* deletion are subunits of receptor degradation machineries such as endosomes and lysosomes. High level of receptor delivery and low level of receptor degradation concomitantly enrich receptors on the membrane and enhance the activity of the receptors that are involved in maintaining stemness, including RTK, Notch1 and Frizzled. Lower lysosomal activity induced by *Qki* deletion also leads to defective mitophagy, which consequently leads to accumulation of damaged mitochondria, high level of ROS, and genomic instability in *Qki*-deficient NSCs. We identified that genomic instability induced by *Qki* deletion led to accumulation of classical glioblastoma-associated mutations such as copy number gains of PDGFR α , Cyclin D1 and Cyclin D3. Taken together, our data suggest that *Qki* loss-induced endolysosomal defects promotes

gliomagenesis through both reducing receptor degradation and inducing genomic instability.

STEM-26. HYPOXIA REGULATES NOTCH TURNOVER IN GLIOMA STEM-LIKE CELLS

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Tumor hypoxia selects for highly malignant cells and is associated with poor patient survival. Glioblastoma is an incurable brain cancer characterized by regions of hypoxia. The Notch pathway is implicated in maintaining glioma stem-like cells (GSCs) in the hypoxic niche, but the underlying mechanisms are unclear. We have identified Vasin as a critical link between hypoxia and Notch signaling in GSCs. Vasin is preferentially induced in GSCs by hypoxia through a HIF1 α /STAT3 co-activator complex. Vasin binds to and stabilizes Notch1 at the cell membrane by reducing its lysosomal degradation. Vasin therefore acts as a switch to augment Notch signaling under hypoxic conditions. In mouse models of glioblastoma, Vasin promotes tumor growth and reduces animal survival. In human IDH1 wt gliomas, Vasin expression inversely correlates with patient survival. We provide mechanistic insight into hypoxic regulation of membranous Notch1 turnover in GSCs and identify Vasin as a prognostic marker and potential therapeutic target.

STEM-27. HDAC3: THE UNIVERSAL TARGET FOR GLIOBLASTOMA?

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BACKGROUND: Histone deacetylase inhibitors (HDACi) are increasingly investigated as potential therapeutics in various cancers, including the most common brain malignancy, glioblastoma multiforme (GBM). The compound MI-192 has demonstrated selective inhibition of subtypes HDAC2 and 3, reportedly involved in tubulin acetylation and mitosis which drive migration and proliferation. **METHODS:** The anti-migratory and anti-proliferative activity of MI-192 was investigated at two different concentrations (1,10 μ M) in 3D invasion assays with spheroids developed from tumour core and edge cell populations of the patient-derived glioblastoma cell line GBM40. In addition, data was also generated using 2D scratch wound assays and immunofluorescence staining for Ki67 (proliferation), Nestin and SOX2 (stemness), Cleaved caspase 3 (apoptosis), HDAC 2 and 3 expression. **RESULTS:** GBM40 core and edge cell tumour populations displayed distinct phenotypes and differential cell marker expression levels. In 2D assays core-derived cells proliferated in close proximity to one another whereas edge cells displayed an invasive phenotype with individual lamellipodia and focal adhesions. SOX2 and nestin expression were altered in both core and edge-cell populations in response to HDAC inhibition, with both markers displaying upregulated expression in edge-derived cells. Core cells displayed significantly decreased SOX2 expression levels at 10 μ M MI-192. 3D spheroids increased in core area size and demonstrated decreased migration at 1 μ M concentrations, with the reverse occurring at 10 μ M. **DISCUSSION:** This study supports the idea that there are two distinct stem cell populations mediated via SOX2 and nestin, which predominate at specific sites within the tumour cell subpopulations. MI-192 may be a potential therapeutic for the clinical management of GBM affecting tubulin acetylation, SOX2 and nestin expression. This activity appears concentration dependent, inducing a proliferative and less invasive state at low concentration, potentially promoting greater tumour susceptibility to radiotherapy. Further studies are required to determine the in vivo effects of this compound in combination with radiation.

STEM-28. DOT1L EPIGENETICALLY REGULATES GBM BRAIN TUMOR STEM CELLS

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The median survival for patients diagnosed with Glioblastoma (GBM) is only 14 months, due to recurrence, despite current treatment options of surgery, radio- and chemo- therapies. Recurrence results from the invasion of GBM cancer cells away from the initial cancerous mass and may be due to brain tumor stem cells (BTSCs) that are resistant to therapy and capable of tumor initiation. Recently, epigenetic-based therapies have become of great interest given that, unlike genomic mutations, epigenetic alterations are potentially reversible. Disrupter of telomeric silencing-1-like (DOT1L) is the