MCAD as a key vulnerability unique to GBM identified by an in vivo functional genomic screen

GBM is the most common and aggressive primary brain cancer, with about 12,000 new diagnoses each year.

Malignant Brain Tumor Statistics, 2021

Not much improvement in survival since 1975 for elders

A functional genomic screen of metabolism genes in an in vivo model using patient-derived GBM cells (GSCs) uncovered importance of enzymes involved in fatty acid oxidation.

Elevated expression of MCAD in patient GBM samples vs. normal brain.

ACADM mRNA levels in glioma subtypes vs. normal brain (TCGA data set).

Immunohistochemistry for MCAD on tissue microarray derived from normal brain and GBM tissue
**MCAD-knockdown dramatically attenuated tumor growth using GSC.8.11 and GSC.6.27.**

Downregulation of MCAD resulted in severe mitochondrial failure in GBM and longer animal survival.

**MCAD-knockdown significantly extended survival time.**

**Lipid accumulation and reactive oxygen species (ROS)-related damage in MCAD-knockdown GSCs**

**Downregulation of MCAD impaired mitochondrial function**

- Oxygen consumption rate significantly decreased in basal respiration and reserve respiratory capacity in ACADM-deleted GSCs.
- Lipid accumulation and reactive oxygen species (ROS)-related damage in MCAD-knockdown GSCs.

GSC.11 xenograft tumor tissues showed lipid accumulation upon ACADM silencing.

MCAD-knockdown GSC.11 partially rescued in fatty acid free medium (left) and by GSH (right).

**Graphs and Images:**
- Graph showing % survival of GSC.8.11 over time with MCAD-knockdown.
- Graph showing ATP levels in GSCs with and without MCAD-knockdown.
- Graph showing number of cells over time with and without MCAD-knockdown.
- Images of GSC xenograft tumors with and without MCAD-knockdown.
Developing potent, selective MCAD inhibitors

**Hits identified by high-throughput screen of 278k compounds based on RF-MS.**

**MCAD structures with screening hits guides small molecule optimization.**

**Example of a partially optimized screening hit with cellular potency < 1 µM.**

- **Properties**
  - Cmpd ID: 75915
  - MCAD IC<sub>50</sub> (nM): 37
  - SCAD IC<sub>50</sub> (nM): >5,600
  - LCAD IC<sub>50</sub> (nM): >5,600
  - VLCAD IC<sub>50</sub> (nM): >5,600
  - MCAD CETSA IC<sub>50</sub> (nM): ~200
  - MCAD OCCT (nM): 540
  - Pampa Pe (x10<sup>-6</sup> cm/s (% rec)): 11(60%)
  - Plasma St. (m/r/d/h t<sub>1/2</sub> min): 330/100/360/99
  - MW/cLogP/TPSA: 459/2.28/116
  - MPO/BBB: 3.83/3.16

- **Example of a partially optimized screening hit with cellular potency < 1 µM.**

  - **Crystal structure of MCAD:Octanoyl-CoA**
    - Octanoyl-CoA (substrate)
    - Flavin adenine dinucleotide (FAD, cofactor)
  - **Cryo-EM structure of MCAD**
  - Refined map at 3.3 Å Using 88k particles

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