SFB 1389 UNITEGLIOBLASTOMA

FOCUS A – TUMOR INTRINSIC MECHANISMS | FOCUS B – TUMOR MICRO-ENVIRONMENT | FOCUS C – TECHNOLOGICAL INNOVATION | FOCUS D – UNITE CORES

A01 – TARGETING TUMOR CELL NETWORKS TO OVERCOME PRIMARY AND ADAPTIVE RESISTANCE IN GLIOBLASTOMA

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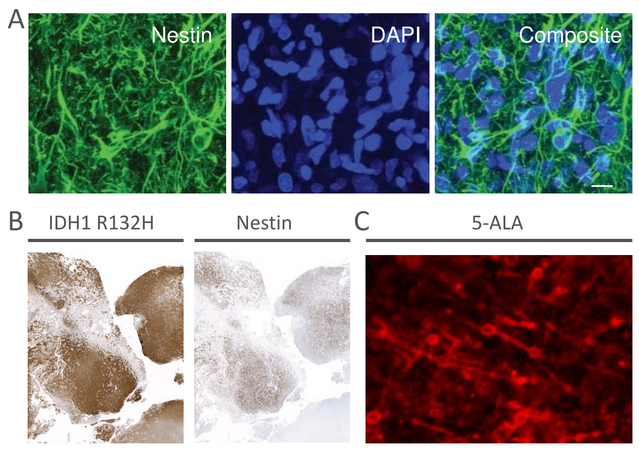
SUMMARY

The main goal of this project is to gain further insights into the biology and relevance of tumor microtubes (TMs), which are thin membrane connections between single glioma cells and an important cellular mechanism of primary and adaptive tumor resistance in glioma animal models. Ultimately, we aim at the development of innovative strategies to inhibit some of their important functions. The specific aims are (1) to understand whether TMs are biomarkers for response to established therapies and adaptive resistance in human glioblastoma, and (2) to develop anti-TM treatments that break resistance to standard glioblastoma therapies.

TASK

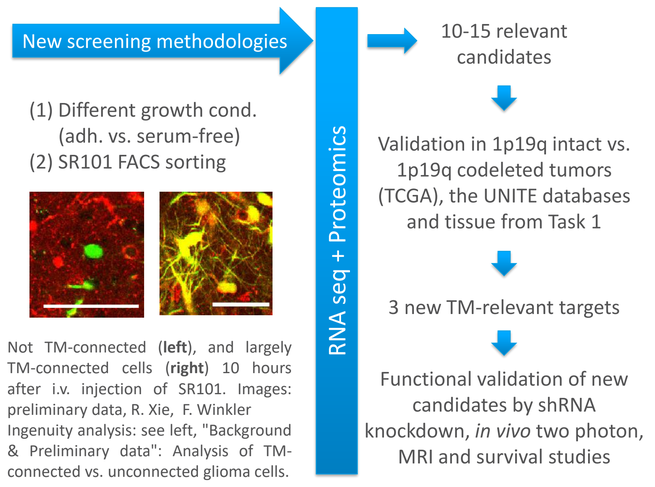
Task 1 - Validation of TMs and their networks as a primary and adaptive mechanism of resistance in human glioblastoma

VISUAL ABSTRACT



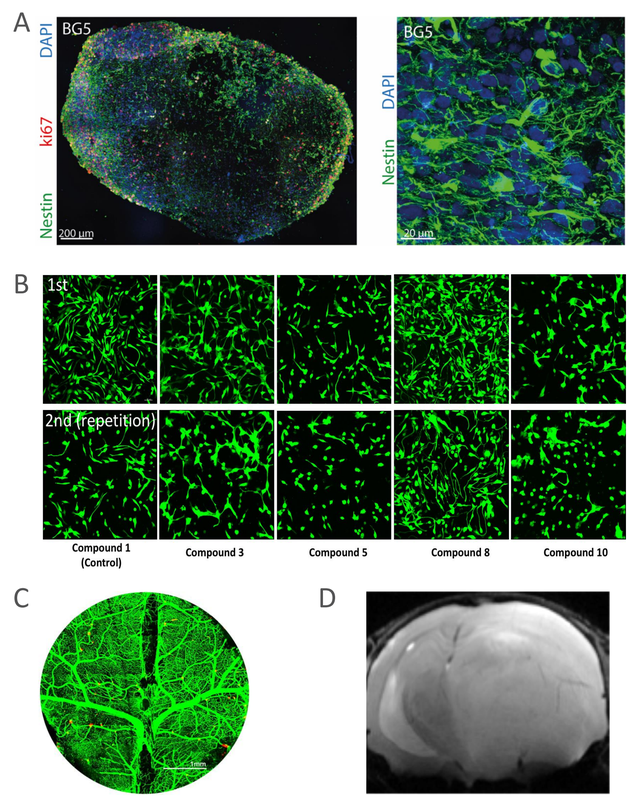
A, Tumor microtube networks in diffuse intrinsic pontine glioma (DIPG) harbouring a typical H3.3 K27M mutation. Autopsy material from a pediatric patient, stained with an anti-nestin antibody that specifically stains tumor cells in this brain region. Tissue provided by M. Monje, Stanford. Image size, 100 x 100 µm. **B**, IDH R132 IHC and Nestin IHC of two adjacent sections show a relevant overlap; costainings will be established as a part of Task1; (unpublished data, V. Venkataramani and F. Winkler). **C**, 5-ALA staining of an acute slice of a glioblastoma patient demonstrating that tumor cells and their TMs can be detected in human tumor samples; (unpublished data, M. Ratliff, V. Venkataramani and F. Winkler).

Task 2 - Identification of new genes involved in TM formation



Not TM-connected (**left**), and largely TM-connected cells (**right**) 10 hours after i.v. injection of SR101. Images: preliminary data, R. Xie, F. Winkler
Ingenuity analysis: see left, "Background & Preliminary data": Analysis of TM-connected vs. unconnected glioma cells.

Task 3 – *In vitro* and *in vivo* screening: effects of selected brain-penetrant compounds on (a) TM morphology and function, (b) glioma growth and resistance



A, Glioma cell networks in a novel brain organoid model. Left, Brain organoid cross section demonstrates the infiltrative tumor growth. Right, Dense tumor cell network formation resembling the network structure found *in vivo* (unpublished, E. Jung and F. Winkler in cooperation with Ph. Koch, Mannheim). **B,** Refined 2D *in vitro* screen (co-culture with astrocyte components) for drug effects on TMs and their networks. Compounds 5 and 10: anti-TM effects, Compound 8: increase in TM-number. Size of every image, 100 x 100 µm (unpublished, E. Jung and F. Winkler). **C, D,** Images acquired by two photon imaging (**C**) and MRI (**D**).

WORKFLOW

- Quantification of TM length, number and interconnectivity; response to therapy in primary/ recurrent patient glioma tissue
- correlation of TM occurrence and therapy resistance (time to progression; OS) in human gliomas
- validation of new relevant genes (Task2) in human samples

- Two combined *in vitro/ in vivo* screening tools of PDX glioblastoma cell lines
- Functional validation of new candidates by gene knockdown, *in vivo* two photon and survival studies

- *In vitro* screen of new compounds (n=100) in 3D co-culture/ brain organoids (selection instructed by new candidates from Task 2)
- *In vivo* screen by two photon microscopy, MRI and mouse survival
- Combination of the most convincing compound and standard therapies (radio- and chemotherapy)

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