PARTNERING OPPORTUNITY

GENETICALLY ENGINEERED HUMAN 3D TUMOR MODELS FOR DISEASE MODELING AND COMPOUND EVALUATION

PARTNERING PROPOSAL

Human organoid tumor models have been generated by introducing oncogenic mutations during cerebral organoid formation via transposon- and CRISPR-Cas9-mediated mutagenesis. Based on clinically relevant mutations the technology has been applied to generate central nervous system primitive neuroectodermal tumor (CNS-PNET)-like and glioblastoma (GBM)-like disease models. In contrast to patient derived tumor models (xenografts, tumor cell lines) this novel platform technology allows to create defined genetic models that contain tumorous and healthy tissue in the same organoid derived from the same human stem cell origin. Such disease models can be cultured over months and are suitable for various applications in the field of human disease modeling, drug discovery and therapy development.

IMBA is actively seeking for **licensing partners** with business interests in the field of **organoid technologies, preclinical testing** or **brain cancer therapies** to exploit the proprietary disease models and/or to expand the technology platform further.

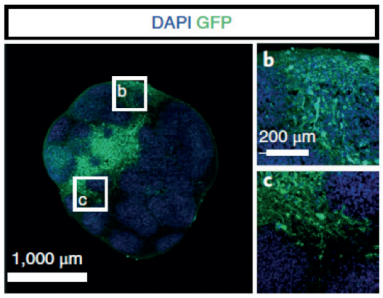
TECHNOLOGY BACKGROUND

Brain tumors are among the most lethal and devastating cancers. Their study is limited by genetic heterogeneity and the incompleteness of available laboratory models. Thus, the etiology of most brain tumors is not well understood. Cell lines derived from human brain tumors cannot effectively model the key aspects of tumorigenesis, including microenvironment contribution, invasiveness, angiogenesis or inflammatory responses. *In vivo* models such as carcinogen-induced or genetically engineered rodent models and xenograft models provide a more accurate experimental system but phenotypic diversity of genetic disorders between species have to be taken into account. In this context human 3D organoid culture models offer an innovative opportunity for human disease modeling and therapy development and complement existing tools.

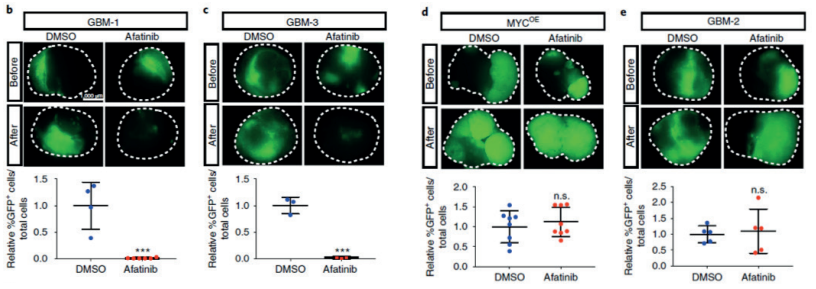
The technology has been used to generate central nervous system primitive neuroectodermal tumor (CNS-PNET)-like and glioblastoma (GBM)-like disease models. To obtain tumor tissue in conjunction with normal tissue only a portion of cells was transfected at the stage of embryonic body formation and visualized by GFP co-transfection. Further growth and differentiation gives rise to disease models that have been designated as neoplastic cerebral organoids (neoCORs) by the IMBA scientists. These proprietary models allow to recapitulate tumorigenesis in culture over months.

To verify that **brain tumor-like organoids resemble distinct brain tumor subtypes** transcriptome analysis, gene clustering and pathway analysis has been performed. Moreover, it has been shown that they retain viability and subtype identity after engraftment in the renal subcapsular region of nude mice. Depended on their mutagenic nature **xenografts showed proliferation**, and for GBMs also tumor invasion into neighboring tissue 6 weeks after implantation.

neoCORs establish valuable complements to existing preclinical models for studying genetic forms of brain carcinogenesis *in vitro*. They constitute novel validation and screening tools for exploratory drug discovery approaches as well as for the development of patient stratification strategies and novel therapies. Tailored and clinically relevant brain tumor models can be generated and any combination of gain- and/or loss-of-function of tumorigenic genes can be introduced by combining transposon-mediated gene insertion for oncogene amplification with CRISPR-Cas9-based mutagenesis of tumor suppressor genes.



Immunofluorescence image of the tumor-normal interface in GBM-1 neoCORs (a), with higher magnification views of highlighted regions.



Immunofluorescence images of various neoCORs and FACS quantification of cells from such neoCORs after Afatinib treatment (b-e). eoCORs are suitable for preclinical compound testing. Immunofluorescence images of organoids and FACS quantification of cells from neoCORs after the indicated treatments.The percentage of GFP+ cells from drug-treated groups was normalized to the percentage of GFP+ cells from DMSO-treated neoCORs. Afatinib decreased the ratio of tumor cells in GBM-1 and GBM-3 organoids (b,c)

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AREAS OF APPLICATION

DRUG EFFICACY TESTING – PROOF OF CONCEPT

- Proof of concept has been demonstrated using the endothelial growth factor receptor (EGFR) inhibitor Afatinib, a lung cancer drug which is currently in clinical trials for treatment for glioblastoma. Different neoCORs (GBMs of subtype 1-3 and MYC overexpressing organoids) have been treated for 40 days and a significantly reduced number of tumor cells for two of the GBM subtypes could be observed, that specifically overexpress EGFR. In contrast, no effect was observed for GBM-subtype-2 organoids or in cells with strong MYC overexpression (MYC^{OE}).
- For **facilitating broader screening approaches** GBM-subtype-1 organoids (GBM-1) were generated that express **firefly luciferase in tumor cells**. They have been used for the analysis of four additional EGFR inhibitors, the experimental drugs Canertibib and Pelitinib, as well as Erlotinib and Gefitinib which are approved for different types of cancers. In this study only Erlotinib showed significantly reduced number of tumor cells, while effects of other inhibitors were minimal for this subtype.

FUTURE DIRECTIONS AND BIOMEDICAL APPLICATIONS

- neoCORs generated from patient-derived induced pluripotent stem cells could be used to investigate the interaction between transformed and non-transformed cells as well as testing the susceptibility of individuals to different combinations of driver mutations or they could give rise to the identification of novel tumor driver genes.
- By generating disease models based on known mutations of brain tumor patients the efficacy of particular treatments for improved patient stratification as well as the combination of therapies could be evaluated.
- neoCORs are limited by their lack of vasculature. Therefore, characteristic features of GBMs such as glomeruloid microvascular proliferations and perivascular palisading necrosis are not observable. The development of appropriate co-culture organoid systems could overcome these limitations and improve the technology further.

REFERENCE

Bian et al. (2018). Genetically engineered cerebral organoids model brain tumor formation. Nature Methods 15, 631-637. *https://doi.org/10.1038/s41592-018-0070-7*

PATENT SITUATION

IMBA filed a European patent application in 2018 followed by a PCT application (WO2019/048689). The application claims the generation of human 3D tumor models and their use in studying disease phenotypes and progression, especially for neoplastic cerebral organoids (neoCORs).

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