Generation and visualization the 3D mice-derived tumor organoids, expressing dcas9-FP probes

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Motivation

Molecular markers do not always correlate with therapeutic response, which highlights the need for the development of universal tools reflecting the tumor's phenotype-genotype profiles.

Functional drug screening in 3D models derived from resected solid tumor tissues represents a more advanced approach compared to studies conducted on 2D cell lines.

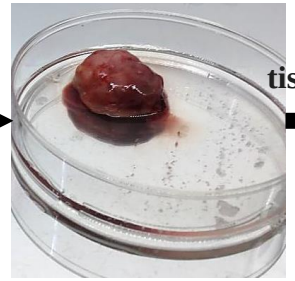
Soft tissue sarcomas are under close attention of researchers and classified as some of the most heterogeneous malignant neoplasms, demonstrating high potential for metastasis and chemoresistance.

The use of fluorescent orthologs (expressing dCas9-FP) in 3D models can contribute to experimental visualization, identification of therapeutic sensitivity, and the study of the cytotoxic effects of drug molecules.

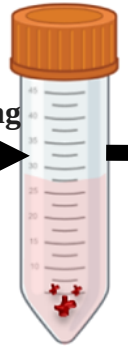
Tissue Processing and Cell Model Establishment

3d tumor sarcosphere derived from surgical resection specimens

surgical resection

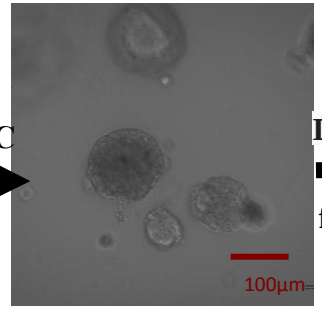


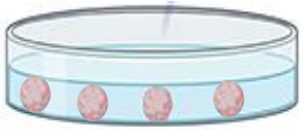
tissue processing



CO^2 5% 37°C

1-2 weeks

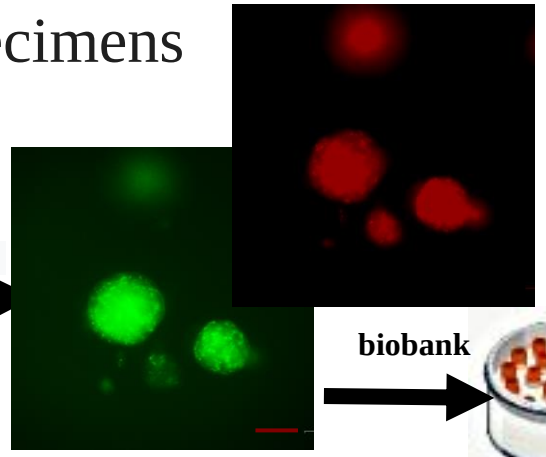




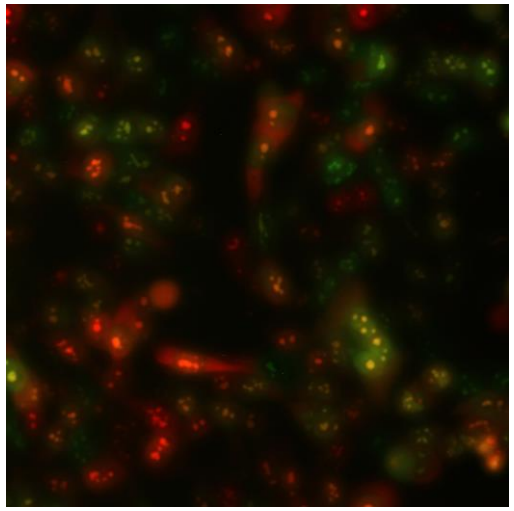
3d tumor sarcosphere

Dox 3-7 days

fluorescence



biobank



The diagram illustrates the process of tissue processing and cell model establishment. It starts with the surgical resection of a tumor, where the extracted tissue is processed and placed in a Petri dish for cultivation. The cells are cultured in a CO_2-enriched environment (5%) at 37°C for 1-2 weeks, forming 3D tumor sarcospheres. Doxycycline (Dox) is then added for 3-7 days to activate fluorescent markers (dCas9-FP), allowing for fluorescence visualization. The final 3D tumor models can be stored in a biobank for future research and experimental studies.

3D Sarcosphere Tumor Models, expressing dcas9-FP

3D tumor organoids are reckoned as the most prominent models to imitating the tumor phenotype and heterogeneity *in vivo*

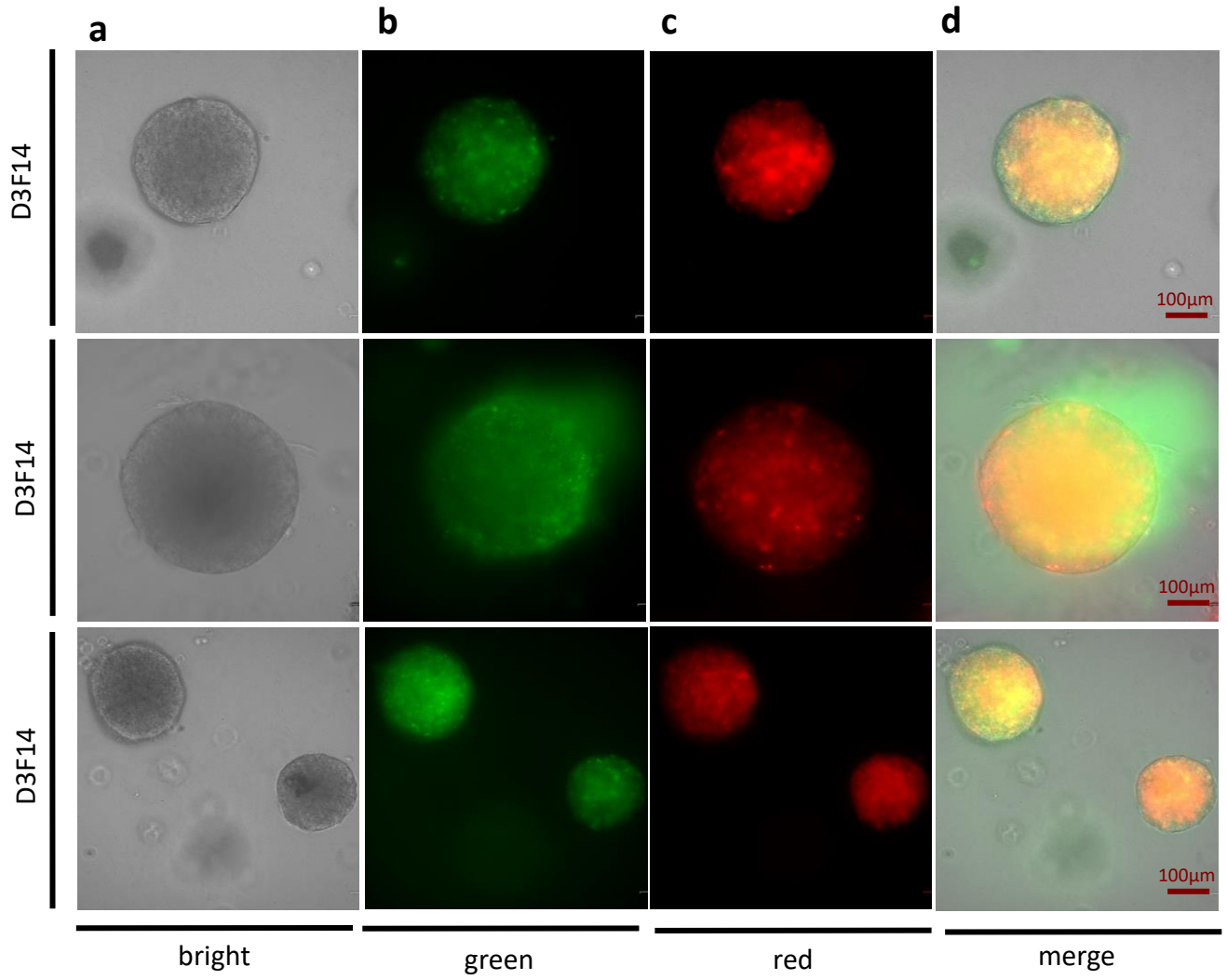
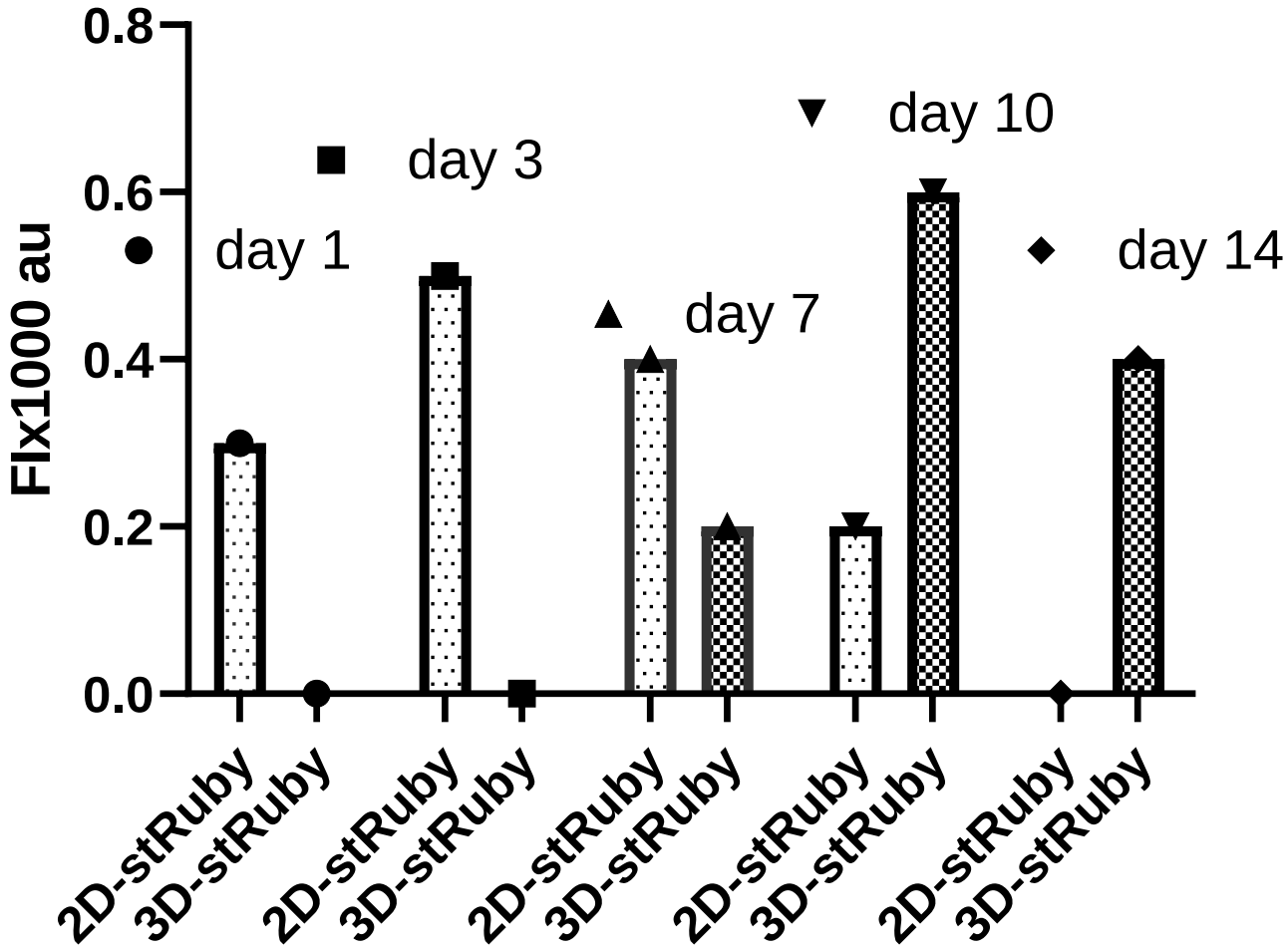


Figure 1. Three-dimensional sarcosphere MNNG/hos expressing dcas9-nmNeonGreen-dcas9-stRuby

(a) Original 3d tumor. sarcosphere in brigthfield ;
(b) Green channel (dcas9-nmNeonGreen)
(c) Red channel (dcas9-stRuby).
(d) Merge (dcas9-nmNeonGreen-dcas9-stRuby).Scale bar: 100 µm

Manifestation of transgene expression (red chimera st-Ruby) in 2D-cell line MNNG/Hos and in 3D tumor organoids, generated from the tumor biopsy of the ones



Conclusions

- 3d tumor osteosarcoma models, expressing dcas9-FP were successfully established
- The genetically encoded probe manifestation differed in 2-D and 3-D tumor models, probably due to tumor microenvironment impact
- According to the literature, the difference in the time of manifestation of gene expression in a primary culture isolated from a tumor may be associated with the processes of reversible methylation and demethylation of gene promoters, in particular the cytomegalovirus promoter of transgene expression.
- This effect is leveled out as macrophages and other cells in the mixed culture , providing a cytokine immune response, die (*Zhang SC et al., Reversible promoter methylation determines fluctuating expression of acute phase proteins (2020*))

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