PDX-derived tumor microtissues as ex vivo human experimental models for evaluating therapeutic responses

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Abstract

The selection of appropriate preclinical models comes always with the major question on how accurately and robustly they can represent the complexity of human disease. Patient-derived xenograft (PDX) models faithfully preserve the biological features and the genetic expression profile of human tumor specimens. However, one limiting aspect of patient-derived models is the replacement of the human host microenvironment by murine stroma within the tumor. Lack of cross-species compatibility compromises the induction of a broad range of signaling pathways that cannot be entirely recapitulated. With our in vitro 3D InSphero™ Tumor Microtissues derived from PDX lines, we provide a relevant physiological environment and a strategy to assess candidate drugs for novel therapeutic approaches.

Charles River Laboratories (CRL) provided InSphero with PDX derived cell suspensions of lung (LXFA 1647, LXFA 677, LXFI 1121), breast (MA2X 2500) and melanoma (MEXF 2106) origin. 3D microtissues were produced in the AKURA™ 96 plate format and characterized over 10 days in culture. Morphology and biomarker phenotype were assessed by histological analysis. Phenotypic resemblance of microtissues was assessed by immunohistochemistry. Growth rate and viability of PDX-derived microtissues were determined by size analysis (cell counter) and ATP assay. To provide a more physiological cancer niche, PDX cells were also co-cultured with tumor-matched cancer associated fibroblasts (CAFs). Furthermore, we assessed the efficacy of specific targeted therapies which were selected on the basis of the distinct molecular signatures of PDX tumor cells.

3D InSphero™ Tumor Microtissues

Xenograft mouse models as vehicle for patient-derived tumor cells provide an efficient way to expand precious human tumor materials, which maintain the entire repertoire of fundamental features for the drug screening in early passages into mice. 3D InSphero™ Tumor Microtissues help to bridge the gap existing between preclinical animal studies and the translation of outcome into clinical applications.

Advantages of Multicellular Spheroids

- Reflect tumor cell heterogeneity
- Retain the original genetic clonality
- Provide the physiological tumor microenvironment (normal/tumor associated stromal)
- Evaluate intercellular interactions beyond intrinsic cancer cell functions
- Enable study of immune cell components (PBMNC, INK and T cells)

Applications in drug efficacy testing

- Cytotoxic drugs or targeted cancer therapies as single agents
- Combinational drug regimens
- Assessment of biological agents (e.g. antibodies)

Validation of in vitro findings

- Media exchange without tissue loss
- Defined relevant volume
- Fully SBS conform and automation compatible

Characterization of in vitro to in vivo correlation

A. PDX-derived Microtissue Morphology

B. PDX-derived Tumor Microtissue Volume

C. PDX-derived 3D Microtissues

D. Biomarker Correlation

E. In vitro PDX

F. Histology

Figure 1: Development, characterization and resemblance of in vitro to in vivo PDX xenografts. Growth of PDX-derived 3D tumor microtissues was monitored through the observation of tumor size. Growth of tumor microtissues over time by DF imaging (A-E). Each model was supplemented with xenograft human stromal cells to recapitulate the human host microenvironment. In addition, the culture medium was kept constant across all multilayer spheroid cultures. Growth curves were used to produce multiple imaging (A) of tumor microtissues at different time points (days 10-15). Further characteristics were based on morphology and standard immunohistochemistry (IHC) diagnostics on histological sections (E). In vitro and in vivo xenografts were selected to represent (A) melanoma (MEXF 2106) and (B) breast (MA2X 2500) models. The IHC of the tumor microtissues was compared with the original tumors (C). Growth of tumor microtissues was characterized by specific tumor marker expression (AKTR, HSC, CRABP1) and Vimentin (E). In vitro expression profiling was correlated with the in vivo staging results (C). The heatmap graphical representation describes the distribution of the relative expression properties in PDX-derived 3D tumor microtissues (E).

In vitro to in vivo drug efficacy correlation

A. Mono-culture

B. Co-culture

Sensitivity of 3D Microtissues toward Vemurafenib comparison to a 3D isogenic assay. Both microtissues in mono and co-culture were treated with 30 nM of Vemurafenib and compared to Control (0 nM). The treatment with Vemurafenib reduces tumor volume and tumor growth both in vitro and in vivo. In vivo bearing MEF 2106 tumors were treated with Vemurafenib (30 μg/kg/day) and the tumor microtissues were treated with 30 nM Vemurafenib. The treatment with Vemurafenib reduces tumor volume and tumor growth both in vitro and in vivo. In vivo bearing MEF 2106 tumors were treated with Vemurafenib (30 μg/kg/day) and the tumor microtissues were treated with 30 nM Vemurafenib. The treatment with Vemurafenib reduces tumor volume and tumor growth both in vitro and in vivo. In vivo bearing MEF 2106 tumors were treated with Vemurafenib (30 μg/kg/day) and the tumor microtissues were treated with 30 nM Vemurafenib. The treatment with Vemurafenib reduces tumor volume and tumor growth both in vitro and in vivo.

Summary

PDX-derived 3D InSphero™ Tumor Microtissues faithfully model the features and heterogeneity of original human tumor specimens.

Further efforts will be focused on employing this platform to establish more complex co-cultures with integration of additional relevant stromal and immune cells, to enable a reliable preclinical translational research of tumor-immune cell interactions. We suggest that 3D InSphero™ PDX models offer a more suitable and robust approach to expedite efficacious assessment of immunomodulators and approval of optimal drug candidates.