High-Throughput Compound Screening with Triple Negative Breast Cancer Organoids

Abstract:

Three-dimensional patient-derived organoids have been demonstrated to largely retain the clinical heterogeneity, genetic characteristics, and drug sensitivity and provide a reliable platform to screen novel druggable targets and new therapeutic agents. However, successful applications of organoids for medical purpose remain very limited. For example, triple negative breast cancer (TNBC) is considered as the most aggressive type of breast cancer with few therapeutic options and poor prognosis. So far, there are no reliable and economic models for target screening and drug discovery in TNBC. Despite complexity and instability of organoid-based assay, Organoid model is providing a promising platform to establish a high-throughput screening (HTS) system. In this work, the scientists of K2 Oncology Co. established 15 TNBC organoids and a TNBC organoid-based 96-well HTS assay to screen potential anti-cancer compounds.

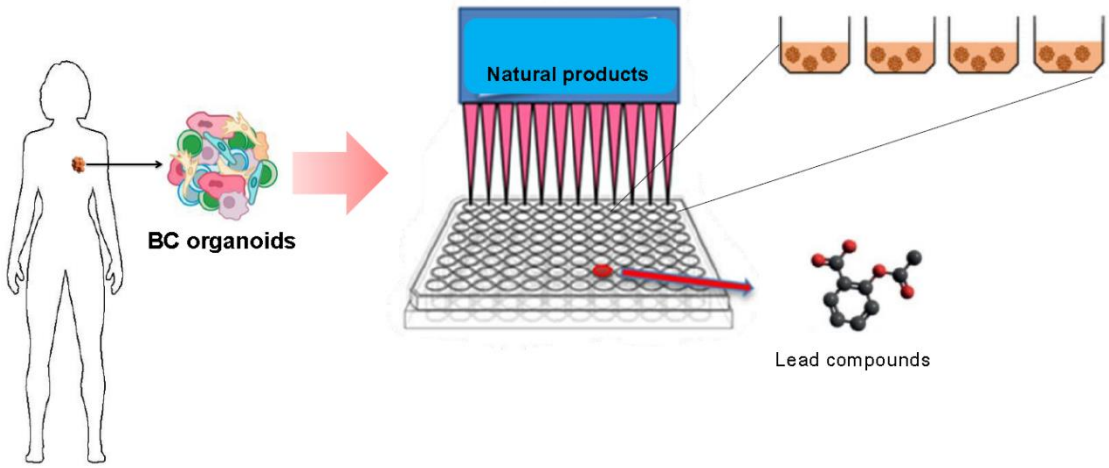

Fig. 1. Schematic diagram for high-throughput **screening of natural compounds in patient-derived organoids**

Culture of TNBC organoids

Recently, patient -derived organoids (PDOs) have been reported to show 100% sensitivity, 93% specificity, 88% positive prediction-value, and 100% negative prediction-value for evaluating response to targeted agents or chemotherapy in patients [1]. In comparison with cancer cell lines, PDOs are reliable model to largely retain tumor characteristics and tumor microenvironment (TME), such as cell-to-cell interactions, cellular heterogeneity, nutrients, oxygen, and chemical gradients of the original tumor (Table 1). We have already established 15 TNBC organoids form surgical tissues or malignant pleural fluid.

Table 1: Comparison of PDOs and cancer cell lines in modeling tumor features.

Types	PDOs	Cancer Cell Lines
nutrients	√	×
oxygen	√	×
chemical gradients	√	×
cell-to-cell interactions	√	√
Original tumor heterogeneity	√	×

It has also been proved that the 3D organoids are more sensitive to drug than cancer cell lines. As shown in Fig. 2A, the same compound is nontoxic in the 2D-cultured cell lines but highly toxic in 3D-cultured cancer organoids. It has been found that leukemic cells cultured in 3D condition were more resistant to drug-induced apoptosis than cells cultured in 2D condition (Fig. 2B) [2].

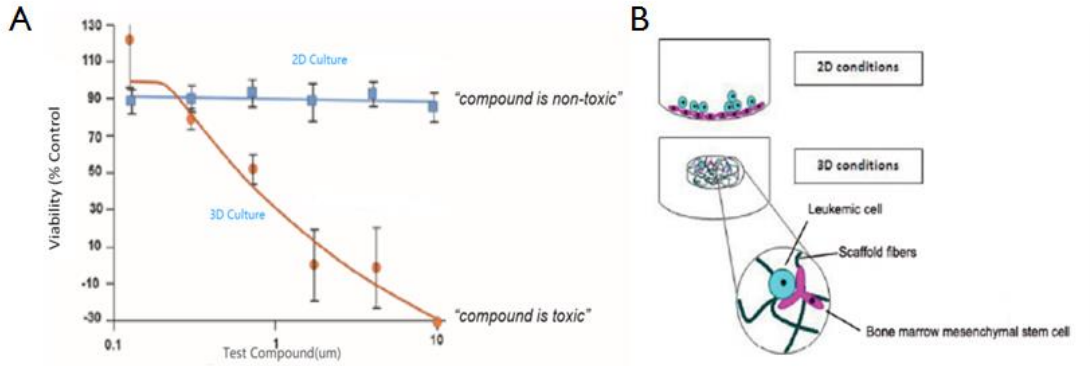

Fig 2. 3D assay *versus* 2D assay in drug sensitivity evaluation.

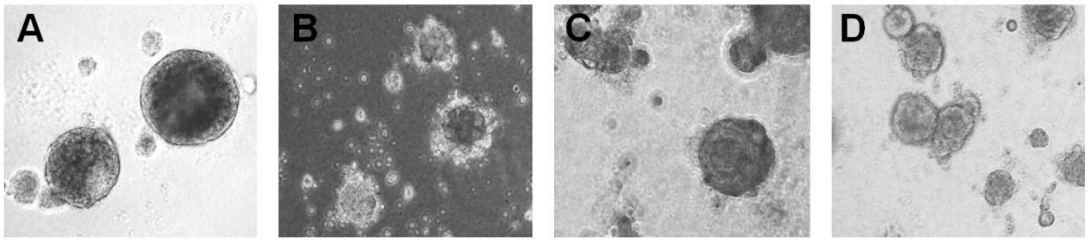

Fig. 3. Bright-field images of breast cancer organoid. (**A-D**) Organoids from patients show different phenotypes under microscope. In early passages, there is obvious heterogeneity in organoids derived from the same patient.

Natural product library

Natural products are small molecules produced naturally by any organism including primary and secondary metabolites. Nature has been a source of medicinal agents for thousands of years, and many based on their use in traditional medicine a large number of modern medicines have been isolated from natural organisms, some of which have been used as traditional medicines for a long time. With the identification of new druggable targets, there are increasing demands to find novel therapeutic agents through screening various chemical pools, including natural products.

For potential screening, we have collected a unique library of 2470 natural products, containing Saccharides, Glycosides, Phenylpropanoids, Quinones, Flavonoids, Terpenoids, Glycosides, Steroids, Alkaloid, Phenols, Acids, and Aldehydes.

Hits of natural products from an organoids phenotypical screening may identify potential bioactive components as lead compounds for new drug development with promising commercial application .

Fig. 5. Natural product library

HTS assay development

High throughput screening is the use of automated equipment to rapidly test thousands to millions of inorganic or orgonic molecules for biological activity in model organisms at the levels of cells, sub-cells, signaling pathways, or molecules. However, about 90% of the promising preclinical drugs have not

been effectively used to treat cancers [3]. There is an urgent need to perform HTS in a reliable culture systems, which could faithfully model the tumor microenvironment (TME) and the heterogeneity of cell types compared to traditional 2D culture systems. To address this requirement, we have developed a panel of patient-derived organoids (PDOs) amenable to screening in a 3D spheroid format on K2 Oncology's cellular HTS platform.

Tumor organoids (Pt-016) were recovered and expanded for 1-2 weeks before plating. Enzyme digested organoids were embedded in an extracellular matrix and subsequently self-organized into organoid structures within 2 days of culture before compound treatment (Fig. 6A).

An endpoint CellTiter-Glo® 3D cell viability assay has been validated as HTS assay. The assay is a homogeneous method to determine the number of viable cells in organoids culture based on the quantitation of intracellular ATP, which is a specific measurement for metabolically active cells. We performed validation of assay robustness and reproducibility via plate uniformity studies (Fig. 6B).

DMSO (0.1% v/v working concentration) was employed as negative control; Staurosporine (1μM) was used as positive control; the values of positive control and negative control were used for normalization and calculation of *Z* factor. Then , the *Z* factor is employed to evaluate the assay quality. In this study, *Z* factor larger than 0.5 will be consider as qualified test (Fig. 5C-D).

Equation：*Z* factor = 1 - (3 * (σ p + σ n) / |(μ p- μ n)|)

The 3D CellTiter-Glo® 3D cell viability assay has passed all relevant validation criteria after assay optimization.

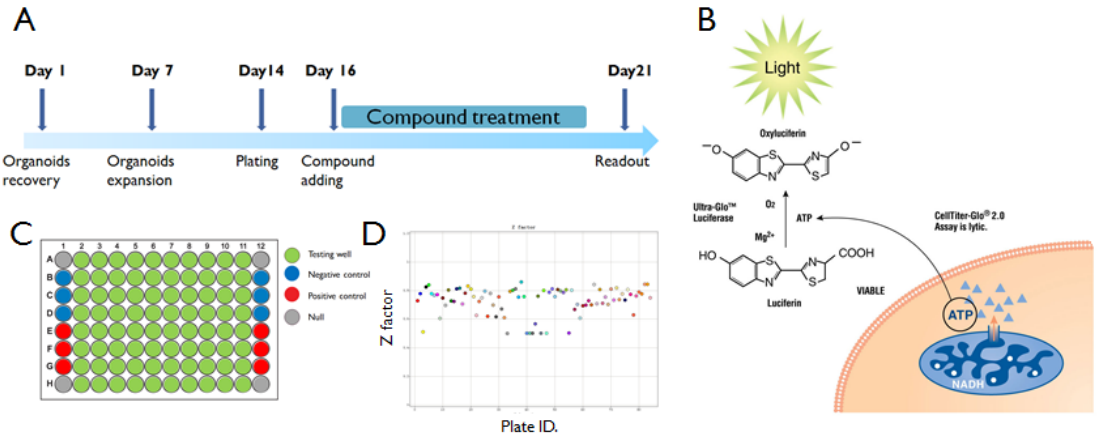

Fig. 6. HTS Screening with TNBC organoids

TNBC organoids Pt-016 was used for HTS due to its fast growth property. We screened a total of 2470 compounds at 1 uM for their ability to suppress cancer cell viability. Of the 2470 compounds, we have identified 23 compounds showing 50% inhibitory effects on Pt-016 viability in comparison with DMSO **(Fig. 7)**.

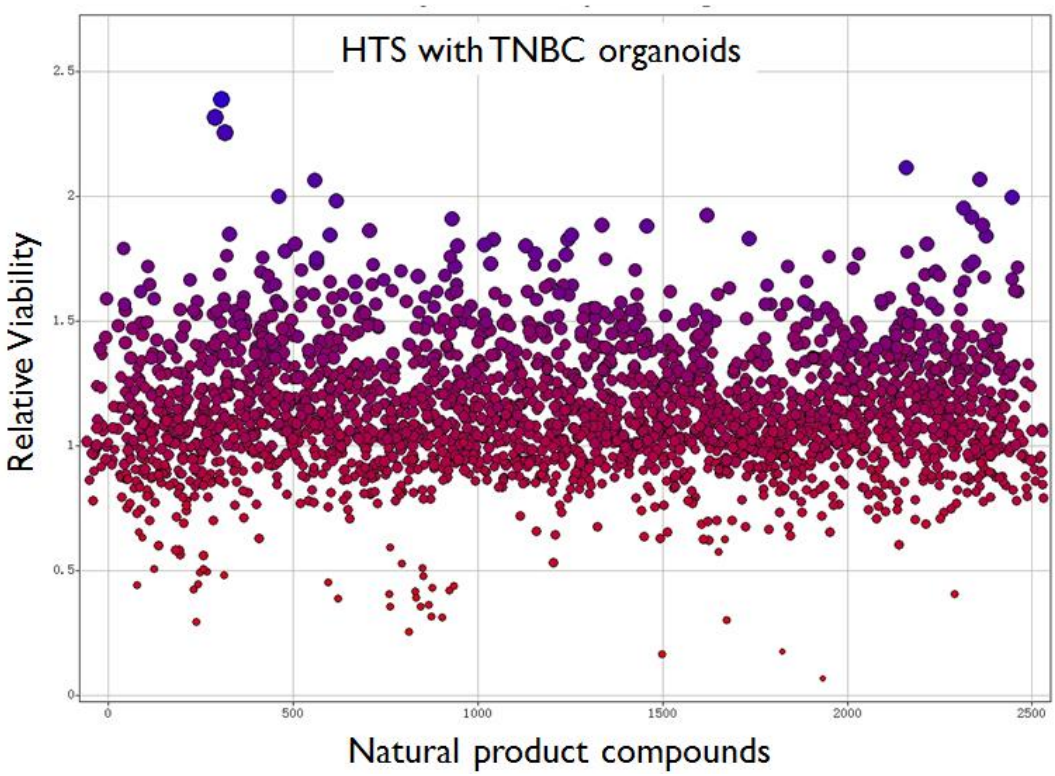

Fig. 7.High-throughput screening with TNBC organoids

Secondary compound validation

Hits from primary screen were identified from one organoid. The sensitivity needs further validation in more TNBC organoids. Therefore, other ten TNBC organoids were developed to further validate the sensitivity of primary hits **(Fig. 8)**. Using organoids from more individuals as the reference effectively increases the accuracy of the prediction.

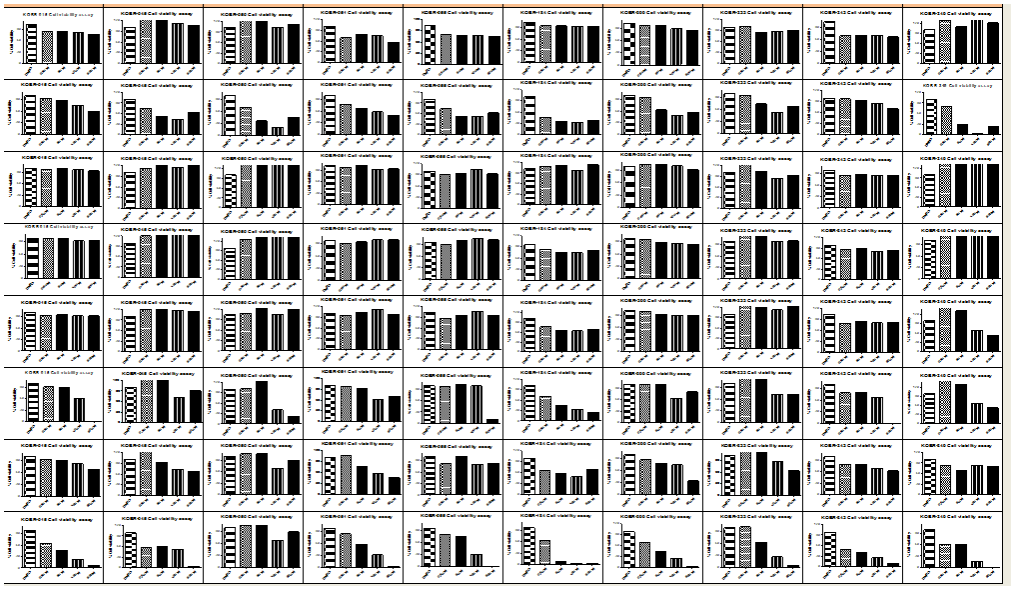

Fig. 8. Hits validation in more TNBC organoids

Conclusion：

As a reliable pre-clinical model, TNBC organoids retain most of oncogenic characteristics, and provide an ideal platform for phenotype screening. Moreover, TNBC organoids can be used to establish a robust in vitro assay for drug sensitivity with more advantages than breast cancer cell lines. Altogether, our results demonstrate the feasibility of applying patient-derived TNBC organoids for high-throughput assays in drug discovery as disease-specific models.

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References

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