Exploiting 3D bioprinted models to assess drug response in Chronic Lymphocytic Leukemia

R. Pinos[1], M. Cellani[1], F. Barbaglio[1], L. Scarfò[3,4], P. Ghia[2,4], C. Scielzo[1]

[1] *Division of Experimental Oncology, Malignant B cells biology and 3D modelling Unit, San Raffaele Hospital (IRCCS), Milan, Italy*

[2] *Division of Experimental Oncology, B cell neoplasia Unit, San Raffaele Hospital (IRCCS), Milan, Italy*

[3] *San Raffaele Hospital (IRCCS), Milan, Italy*

[4] *Vita-Salute San Raffaele University, Milan, Italy*

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INTRODUCTION

Inter-individual diversity is driving clinical approaches through a patient-specific treatment strategy almost in every medical field, as cancer. However, conventional 2D culture models are still widely used to test their effectiveness, thus not allowing to mirror pathophysiological complexity, particularly in terms of prediction of response to therapy. In our laboratory, we set up a 3D bioprinted model to assess the response to chemotherapeutic agent and target therapies in Chronic Lymphocytic Leukemia (CLL) cell lines and primary cells, with and without the addition of the tumor microenvironment. Furthermore, we used dynamic culture settings to increase the complexity of our platform, also to deliver drugs in a more physiological way.

MATERIALS AND METHODS

CLL line cells (MEC1) were printed in an alginate/gelatine-based bioink [1] while primary cells were printed in CELLINK Laminink411. In the microenvironment model, stromal cells were printed in GelXA Laminink411 and MEC1 in the bioink mentioned above. All the scaffolds were printed using a CELLINK BioX bioprinter. Cell viability and gene expression analysis were performed on control and treated 2D and 3D samples after 72 hours of treatment. Confocal imaging was used to assess cells compartimentalization in microenvironment-laden 3D models. Dynamic delivery of drugs was guaranteed by a peristaltic pump and a 3D printed custom-made bioreactor.

RESULTS AND DISCUSSION

As previously demonstrated by our group, 3D bioprinting allows the establishment of a long-term culture for both CLL cell lines and primary cells, thus enabling to investigate their phenotype and behaviour at distant time-points. Molecular diversity was demonstrated by RNAseq analysis in 3D bioprinted MEC1 cells, compared to the conventional 2D counterpart, resulting in the up-regulation of genes involved in cell adaptation and survival (e.g. SELL and BCL2) [2]. On top of that, we exploited our 3D platforms to assess the response to Fludarabine in 2D and 3D. Specifically, 3D bioprinted MEC1 cells showed higher resistance to therapy (~80% of viable cells), if compared to 2D (~20% of viable cells) 72 hours after treatment. Moreover, drug delivery in dynamic settings caused a drop in cell viability in 3D (~40% of viable cells). Concerning primary cells, we need to use a dose 30 times higher in 3D in order to obtain the same effect of treatment in 2D (~20% of viable cells). Overall, these data suggest a higher resistance of 3D bioprinted cells if compared to 2D, supporting our findings on the molecular profiling. Indeed, by RNAseq analysis 3D bioprinted CLL cells showed upregulation of genes involved in mechanism of resistance such as AICDA and IL2RA expression, if compared to 2D. In parallel, we set up a multi-material and multi-cellular bioprinting strategy to engineer a microenvironment-laden CLL 3D model, since bone marrow stromal cells, are known to affect CLL cells survival and proliferation [3]. Confocal images showed precise compartimentalization of printed cells, as well as heterotypic cell interactions. Further experiments will be performed to dissect the role of microenvironmental cells in drug response in advanced *in vitro* models.

CONCLUSIONS

3D bioprinted CLL cell lines and primary cells showed increased resistance to a standard chemotherapeutic agent and upregulation of genes potentially involved in this mechanism, if compared to 2D, even in case of dynamic delivery of the drug. We are currently testing the effect of target therapies such as the BCL2 inhibitor Venetoclax. Preliminary data show the feasibility to test drugs in the presence of a relevant microenvironment, adding another layer of complexity to the system. The established platform could be useful to test target and combinatorial therapeutic approaches directly on patients' cells.

REFERENCES

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