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Setting a 3D model to study Chronic Lymphocytic Leukemia

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OBJECTIVES

This project aims to set up a 3D model to study how the tumour microenvironment of Chronic Lymphocytic Leukaemia (CLL) cells may affect their response to specific drugs used in cancer therapy.

CONCLUSIONS

- The gel provides a protection to CLL cells at basal conditions and supports a higher recovery after 28 days of culture.
- Cells in 3D cultures are more resistant to PRIMA-1^{Met} if compared to 2D cultures after treatment with the same concentration of molecule.
- 3D conditions seems to induce a general antiapoptotic behavior in the cells, even after treatment with high concentration of molecule.

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INTRODUCTION

Chronic Lymphocytic Leukaemia (CLL) is a type of B-cell malignance characterized by a high number of interactions between clonal cells and those in the surrounding environment.[1]

The tumour microenvironment itself may affect the response to therapy.

For many years, cancer studies have been carried out on traditional 2-dimensional (2D) cell cultures, however this model does not allow to reproduce a high level of complexity.

3D systems are becoming promising tools to recreate more physiological cells conditions; however, haematological cancers are poorly characterized in this sense.[2]

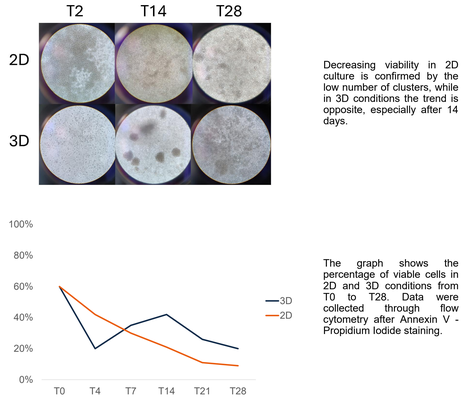
METHODS

- Cell culture: MEC-1 cells (CLL cell line carrying a mutated *TP53* gene) were cultured in 2D and 3D conditions by using VitroGel Hydrogel® Matrix as scaffold.
- Treatment: PRIMA-1^{Met}, a p53-refolding and pro-oxidant molecule, was used at 5μM and 10μM to treat cells both in 2D and 3D conditions.
- Cell viability was measured through flow cytometry (Annexin V – Propidium Iodide staining) and trypan blue exclusion.
- Apoptotic levels were evaluated by qPCR and western blot analysis of the main target proteins.

RESULTS

- 3D conditions promote cells growth more effectively than 2D cultures (**Fig. 1a**) and have a higher recovery over time after 28 days of culture (**Fig. 1b**).
- In 3D conditions there is an increase in Bcl-2 and PARP (but not the cleaved form) after 21 and 28 days of culture and this suggests a general antiapoptotic behaviour (**Fig. 2**).
- Targets related to the autophagic pathway confirmed the antiapoptotic trend, with an increase in p62 and a decrease in LC3-II in 3D conditions at 21 and 28 days of culture (**Fig. 3**).
- The treatment with PRIMA-1^{Met} at different concentrations did not increase the number of apoptotic cells when compared the treatment to the control in 3D conditions, whereas in 2D cultures the molecule cause a decrease in the viability (**Fig. 4**).
- qPCR analysis highlights an increase in Bcl-2 levels following rising concentrations of PRIMA-1^{Met} in 3D conditions and the opposite trend was observed again in 2D cultures (**Fig. 5**).

Figure 1. Cell viability is promoted in 3D conditions.



Decreasing viability in 2D culture is confirmed by the low number of clusters, while in 3D conditions the trend is opposite, especially after 14 days.

The graph shows the percentage of viable cells in 2D and 3D conditions from T0 to T28. Data were collected through flow cytometry after Annexin V - Propidium Iodide staining.

Figure 2. Antiapoptotic behaviour in 3D cultures.

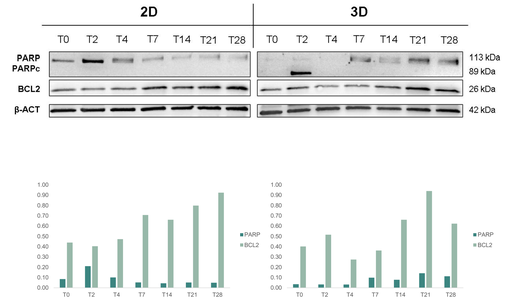


Figure 3. Anti-autophagic trend in 3D cultures.

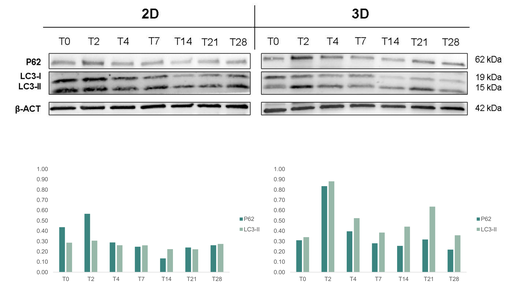
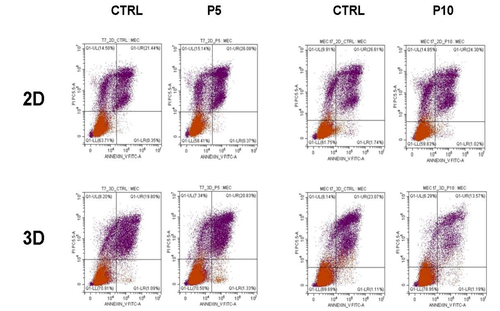
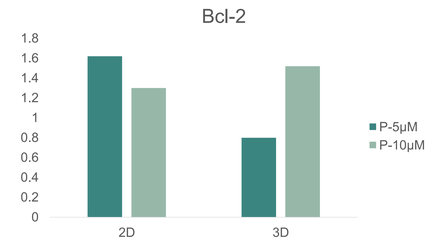


Figure 4. 3D conditions make CLL cells more resistant to treatment.



Determination of cell viability as the percentage of double Annexin V – Propidium Iodide negative cells (red dots). In 3D conditions the percentage of viable cells does not change after treatment with PRIMA-1^{Met} at 5μM and 10μM if compared to control cells. In 2D cultures the treatment impairs the general viability instead.

Figure 5. Antiapoptotic response to treatment in 3D conditions and opposite trend in 2D culture.



Bcl-2 levels were determined through qPCR.analysis. In 3D conditions the increasing concentration of PRIMA-1^{Met} is related to a higher expression of the antiapoptotic protein Bcl-2, whereas the same treatment leads to pro-apoptotic behaviour in 2D cultures.

REFERENCES

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2. Scielzo C et al. 2022, Modeling the Leukemia Microenviroment In Vitro.

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