P1251 BONE-BASED 3D SCAFFOLD AS AN IN-VITRO MODEL OF MICROENVIRONMENT-LYMPHOMA INTERACTION

Topic: 20. Lymphoma Biology & Translational Research

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Background:
Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL) and it accounts for about 30-40% of new NHL cases annually. Although immunochemotherapy is highly effective in DLBCL, yet about 30% of patients will relapse or exhibit refractory disease (r/r DLBCL). Moreover, the presence of bone marrow (BM) involvement in those patients is associated with a higher risk of aggressive features and worse outcome, likely due to the interaction between neoplastic cells and BM tumor microenvironment (TME).

Aims:
The TME is a complex network in which a multitude of cell types interact with an intricate web of macromolecules, the extracellular matrix (ECM). In order to provide a tool for tumor cells and TME interactions studies, we aimed to develop an innovative and versatile three-dimensional (3D) in-vitro model which accounts for the diverse components of TME. The 3D model will be useful to highlight mechanisms behind drug resistance and to test patient-specific therapies.

Methods:
The 3D in-vitro model was developed starting from human decellularized femoral bone fragments, isolated from the trabecular zone, as structural 3D scaffold and biological ECM provider. Specific seeding techniques, together with the construction of a PDMS device, were developed to adapt and expand MSC and DLBCL cells in the scaffold. Adhesion and growth capability of OCI-LY18 DLBCL cell were evaluated in decellularized scaffold (ECM model) and in BM-MSC recellularized one (ECM/MSC model). 3D spatial configuration of the models was studied by histological analysis, confocal microscopy and two photon techniques which allowed the 3D digital reproduction of the model structure. 3D models underwent doxorubicin treatment and viability of DLBCL cells was measured by Annexin V assay and compared to canonical cultures.

Results:
Both HS-5 cell line and BM-MSC effectively adhered to the bone scaffold without synthetic matrix addition. Moreover, the PDMS device we developed accelerate cell adaptation and homogeneous recellularization.

3D confocal microscopy and histological analysis of recellularized model showed the generation of MSC niches which fostered growth and adaptation of lymphoma cells. Both MSC and ECM were effective in hosting tumor cells. Interestingly, neoplastic cells were able to strongly adhere, adapt and grown not only in ECM/MSC model but also to the bare ECM scaffold, suggesting an active interaction between OCI-LY18 and the ECM. Both MSC and lymphoma cells maintained viability and growth capacity when harvested from the scaffold and readapted to canonical 2D cultures.

We found that chemosensitivity to doxorubicin was hampered when neoplastic cells were grown in ECM and ECM/MSC model. Results showed that the levels of apoptosis were significantly lower if compared to conventional...
2D cultures (Figure. Statistical analysis: non parametric t-test; *=0.06; **= p<0.005; ****= p<0.0001; n=4). Moreover, the presence of BM-MSC in the 3D model increased OCI-LY18 resistance to apoptosis both in adherent and in non-adherent cells. Interestingly, the protective effect was not provided from BM-MSC in 2D co-culture in those conditions.

**Summary/Conclusion:**

The 3D bone-based scaffold model we proposed is a powerful tool for exploring cell-cell and cell-ECM interaction in the setting of DLBCL. By recreating a 3D microenvironment, the model may help to clarify the mechanisms behind drug resistance in r/r DLBCL. Nevertheless, this approach may be exploited to create patient-specific models in order to improve personalized therapies.