ImmunoTools IT-Box-139 Award 2012

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Study of the effect of the AML microenvironment on drug resistance of leukemic stem cells.

Acute myeloid leukaemia (AML) is a disorder of early haematopoietic progenitor cells. AMLs often relapse (70-90% of cases) despite initial disease remissions induced in 60-80% of cases treated with conventional chemotherapeutics. Treatment of the relapsed disease is a major challenge. The mechanisms for AML relapse is related to resistance to treatment of AML cells residing in potential sanctuary sites in the bone marrow (BM). It has become evident that the currently used AML model systems, such as established AML cell lines primary AML blasts maintained in standard cell culture poorly represent in vivo AML. The main problems are the lack of stromal cell-AML cell-cell interaction, extracellular matrix (ECM)-AML interaction and paracrine cell-cell communication in these cultures. Additionally, the oxygen tension ([O2]) is very likely to be too high in the ex vivo culture in comparison to the BM, even if taken into account that BM vascularisation increases in AML.
Due to these differences, treatment strategies identified in these models repeatedly fail in vivo tests. The current animal models much better represent the in vivo characteristics of AML and these models provided essential insights into AML development and progression. However, they are very expensive, 20-30% of primary AMLs do not engraft and the development of AML takes several weeks.

The purpose of my PhD is to establish a new model system that could incorporate the advantages of the ex vivo and in vivo animal models that I believe would have a high impact and could greatly advance AML research. This model is a 3D culture system consisting of fibronectin-based extracellular matrix, mesenchymal stromal cells, endothelial cells and AML blasts. Recent results found that in such a culture, the AML cells retain viability much longer than in the standard culture conditions due to the presence of the leukaemia cell microenvironment, such as cell-cell interactions, AML-ECM interactions, paracrine factors and reduced oxygen tension. In order to proceed with this model we isolate primary AML cells from BM using Ficoll gradient centrifugation and seeded on fibronectin coated MSC-endothelial cell feeder layer. Our current goal is to test the:

1. Long term viability and proliferation of the AML blasts
2. The maintenance of the original phenotype of the AML cells (to make sure no AML blast subtypes get lost during the culture)
3. The presence and survival of leukemic stem cells.

The main technique for these studies is based on immunostaining of cell surface markers and their detection with flow cytometry.
I would like to use the IT box to optimise many of these immunostaining techniques. First of all, the Annexin V available in the kit would be used to measure the long term survival of the AML blasts.
Secondly, the IT box would be great for optimising the AML phenotype determination. There are typical cell surface markers for the different subtypes of AML, including: CD2, CD13, CD14, CD15, CD19, CD33, CD34, CD38, CD45, CD54, CD61 and HLA-DR. Nearly all of these antibodies are present in the IT box and many of the antibodies are available with different fluorochrome tags, enabling multicolour detection.
Finally, antibodies from the IT box could be used to set up the detection of the leukemic stem cell population. For this experiment, CD19, CD38, CD44 antibodies in combination with our own CD34 and CD123 antibodies would be used. Once the experiments are optimised, the subset of antibodies required could be purchased in larger quantities.

ImmunoTools IT-Box-139 for Mairead Reidy include 100 antibodies

FITC - conjugated anti-human CD1a, CD3, CD4, CD5, CD6, CD7, CD8, CD14, CD15, CD16, CD19, CD21, CD25, CD29, CD35, CD36, CD41a, CD42b, CD45, CD45RA, CD45RB, CD45RO, CD49d, CD53, CD57, CD61, CD63, CD80, CD86, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V
PE - conjugated anti-human CD3, CD4, CD8, CD11b, CD15, CD14, CD18, CD19, CD20, CD21, CD22, CD31, CD33, CD38, CD40, CD45, CD45RB, CD50, CD52, CD56, CD58, CD62p, CD72, CD95, CD105, CD147, CD177, CD235a, HLA-ABC, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V
PE/Dy647 -tandem conjugated anti-human CD3, CD4, CD8, CD14, CD19, CD20, CD25, CD54
APC -conjugated anti-human CD2, CD3, CD4, CD8, CD10, CD11a, CD11c, CD14, CD16, CD27, CD37, CD42b, CD44, CD45, CD59, CD62L, CD69, CD71, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

DETAILS