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BOOK OF ABSTRACTS



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DEVELOPMENT OF AN *IN VITRO* PLATFORM TO DETECT TUMORIGENIC EVENTS IN HUMAN HAEMATOPOIETIC STEM CELLS (HHSCS)

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Introduction

Allogeneic haematopoietic stem cell transplant (HSCT) is a curative treatment for a number of diseases such as sickle cell anaemia, haemophilia, thalassemia and severe combined immunodeficiency. The treatments require patients to undergo lifelong pharmacological immunosuppression, and appropriate donors need to be identified to prevent adverse immune responses. In recent years, the use of gene-edited human haematopoietic stem cells (GE-hHSCs) has emerged as a solution [1]. However, gene editing can be accompanied by unwanted events that may lead to cancer [2]. For this reason, a system for efficiently detecting the generation of tumorigenic events is greatly needed.

Methods

A novel millifluidic optically-accessible bioreactor (MOAB S.r.l., Milano, Italy) [3] was functionalized with a silk fibroin scaffold resembling the physical bone marrow microenvironment and subsequently perfused with an appropriate culture medium. Human CD34⁺ HSCs or CD4⁺ lymphocytes were grown inside, alone, or in the presence of tumour cells. The expansion of specific populations was tested up to three months after culture start. In a follow-up, genetically modified CD34⁺ cells will be subsequently tested for the appearance of clonal expansion.

Results

Long term culture of primary, human, normal CD34⁺ or CD4⁺ cells was achieved at time-points up to three months. In these conditions, co-culture with tumour cells allowed us to detect the expansion of oncogenic events. Analytical assays, i.e. confocal microscopy and Fluorescence-Activated Cell Sorting (FACS) analyses, were optimized to efficiently detect the appearance of tumour cells. We defined a tumorigenic index of the system as the minimal number of tumour cells, loaded in the coculture system, that could be detected. As little as 100 tumour cells were clearly detected after three months of bioreactor culture. Current experiments are aiming at detecting as little as one tumour cell and/or the emergence of clonogenic CD34⁺ tumour cells.

Discussion

We validated an animal-free millifluidic platform, able to expand primary human cells and allow for the detection of rare tumorigenic events. The sensitivity of the system is currently being expanded by the use of state-of-the-art technologies, such as single-cell RNAseq. This model may allow the detection of rare tumorigenic events due to genetic editing in a reliable, cost-effective and animal-free setting.

References

1. Wu et al, Nature Medicine, 25:776–783, 2019.
2. Haapaniemi et al, Nature Medicine 24:927–930, 2018.
3. Ene-Iordache et al, Front. Bioeng. Biotechnol., 9, 2021.

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