Cytokine Profiling for T Cell Immunotherapy Assays

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Abstract

The cytokine profile of liver cancer - T cell - monocyte triculture in AIM DAX-1 3D Cell Culture Chips is determined using AYOXXA Lunaris™ 11-Plex Cytokine Kit. Supernatant collected from the chips was used for the assay. The utilized sample volume was only 3 µl per replicate for the quantification of 11 different cytokines in every sample.

Introduction

Adoptive cell immunotherapy uses engineered immune cells, such as T cells to target cancer cells in the human body [1]. As the activities of immune cells from different patients are bound to vary, the quality control becomes a critical step in ensuring the success of adoptive cell immunotherapy [2]. Previous 2D assays for quantification of T cell killing efficiency are oversimplified where the ability of T cells to migrate within a tumor microenvironment is often driven by gravity instead of chemotaxis. [3, 4].

By using AIM 3D Cell Culture Chips, we are now able to address this issue. The platform consists of a hydrogel channel and two flanking media channels, where T cells have to actively migrate into the 3D hydrogel channel to locate cancer cells (target) before killing them. As reported earlier, cytokine profiles differ significantly between 2D and 3D cell cultures [3]. Hence, the AYOXXA Lunaris™ 11-Plex Cytokine Kit was used to assess the variation of cytokine signatures upon silencing of the PD-1 receptor on engineered T cells, with or without the presence of monocytes in the hydrogel.

The AYOXXA Lunaris™ Technology is based on a planar microarray of immobilized microbeads coated with antibodies for the detection of different analytes. Signals emitted from microbeads with bound targets are imaged by fluorescent microscopy and then identified and quantified based on known bead locations.

Materials & Methods

Culture Cells in AIM 3D Cell Culture Chips

HEPG2-PreS1 and monocytes cell suspension was prepared at 2.5 M cells/ml and 4.0 M cells/ml respectively in 2.5 mg/ml of collagen type I solution. The collagen solution with embedded cells was filled into the gel channel. Once polymerized, T cell culture medium was injected into the media channels. The medium was supplemented with live/dead markers (Draq7), and the old medium in the chips was replaced 24 h after seeding cancer cells. shRNA-PD-1-silenced or shRNA-control T cells were seeded into the media channels at 6 M cells/ml. High content imaging (Opera Phoenix, Perkin Elmer) was used to determine the live/dead ratio of cancer cells which indicates the killing efficiency of T cells. The supernatant was collected 24 h after the seeding of T cells and frozen at -80 °C until subsequent AYOXXA Lunaris™ assay.

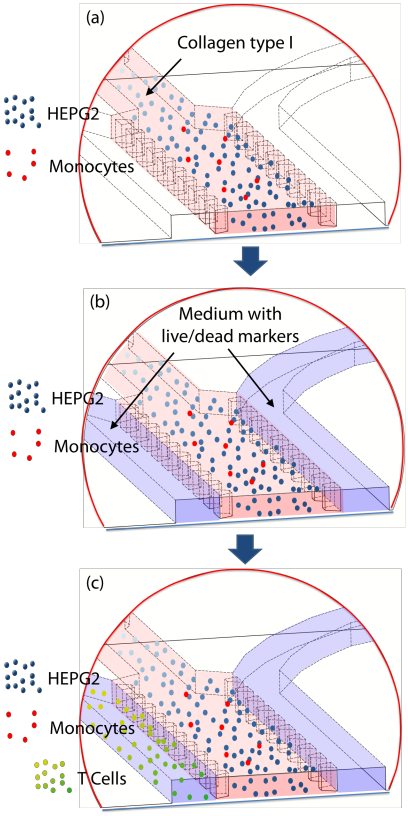

Figure 1 A schematic drawing to illustrate the workflow of (a) seeding HEPG2 cancer cells and monocytes

in the hydrogel channel; (b) filling medium with live/dead markers in the media channels; (c) seeding T cells in one of the media channels.

AYOXXA Lunaris™ Assay

After thawing, 3 µl of supernatant were diluted with 3 µl of Assay Diluent and incubated for 3 h on the biochip. After subsequent steps of washing, antibody incubation, fluorophore incubation and drying, the biochips were analyzed on the dedicated reader. Standard curves unique for each analyte enabled translation of mean fluorescent intensity raw data into concentration values in the pg/ml range.

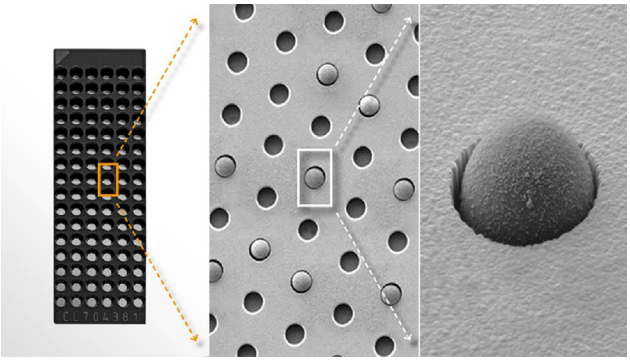

Figure 2 The AYOXXA Lunaris™ 96 well Biochip in the 384 pitch format (left-most) and two SEM images of the antibody-coated microbeads that are immobilized in the cavities of the microarray.

Results

The outcome of the AYOXXA Lunaris™ assay enabled the quantitative assessment of cytokines in the tested samples. Most notably, the presence of CD 14+ monocytes increased the levels of IL-8 and made it more dominant in the cytokine profiles in all groups. CD14+ monocytes also attenuate the levels of IL-5 and the attenuation effects were most obvious in the groups with engineered T cells that can effectively recognize HBV infected HEPG2 in the hydrogel. Besides, CD14+ monocytes also elicit the secretion of IL-6 in the groups with engineered T cells. Engineered T cells also increase the expression of IL-5 as compared to the non-engineered T cells (Mock). And the expression of IL-5 can be further augmented if PD-1 is silenced in the engineered T cells. High levels of IL-2 were detected in all groups as IL-2 was added exogenously to stimulate T cells. The presence of IL-5 also correlates to the killing efficiency of T cells very well where the group with engineered T cells, silenced-PD-1 receptor and the absence of CD14+ monocytes yielded highest killing efficiency and showed the highest proportion of IL-5 in its cytokine profile.

Taken together, the unique combination of the AIM Biotech 3D Cell Culture Chip and the AYOXXA Lunaris™ Multiplex Assay enabled a close look on the underlying mechanism for the T cell killing efficiency and allows to pinpoint critical cytokines signatures.

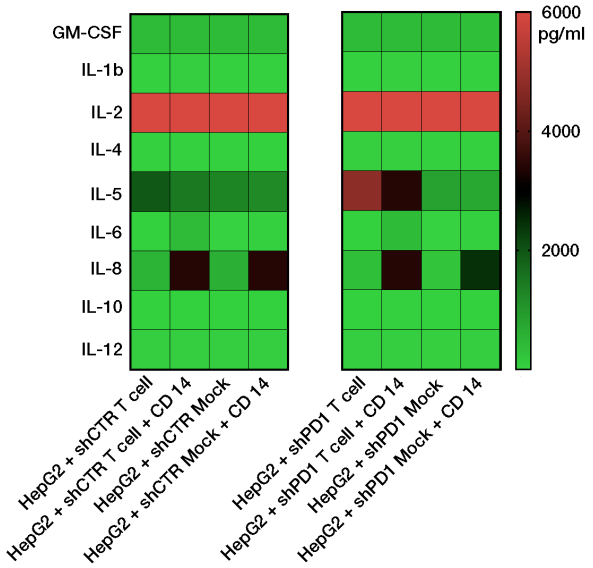

Figure 3 Heatmap of the cytokine profiles. shCTR T cell: engineered T cells transduced with control shRNA; shCTR Mock: non-engineered T cells transduced with control shRNA; shPD1 T cell: engineered T cells transduced with PD-1 shRNA; shPD1 Mock: non-engineered T cells transduced with PD-1 shRNA.

Please visit www.AIMBiotech.com for more information

References

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