Holographic Evaluations of Morphological and Motility Changes in Macrophages in Response to Polarization Stimuli

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ABSTRACT

The tumor microenvironment consists of several cell types including inflammatory cells such as macrophages, which perform critical functions to affect tumorigenesis (1). The macrophages found in the tumor microenvironment are affected by a range of chemo-stimulants, which enable them to exist in a range of different phenotypes broadly classified as the M1 and M2 phenotypes. Although the effect of macrophages post-stimulation has been critically investigated, very little is known about the morphological alterations these cells undergo post chemo-stimulation and in the presence of cancer cells. Thus the aim of our current investigation was to understand macrophage phenotypic changes using the HoloMonitor M4, post stimulation.

INTRODUCTION

The tumor microenvironment consists of several cells including cancer cells and stromal cells (fibroblasts and inflammatory cells) embedded in extracellular matrix and nourished by a vascular network. Amongst the inflammatory cells, macrophages are critical mediators of tumorigenesis. Normally, macrophages have a ubiquitous distribution in organ types, and a wide range of tasks, including bacterial suppression, tumor suppression, immune-stimulation, tissue repair, angiogenesis, and general cellular debris phagocytosis. Their behavior and activation is controlled by a large number of chemo-stimulants, and broadly are classified into two polarization groups, M1 (classically activated macrophages, showing pro-inflammatory characteristics) and M2 (alternatively activated macrophages, showing anti-inflammatory characteristics) (2). M1 macrophages enable the release of cytokines and chemokines that induce bacterial suppression, tumor suppression and immune stimulation. On the other hand the M2 state induces tissue repair and angiogenesis. In the tumor microenvironment this translates into tumor promotion and metastasis (3). Due to the plethora of macrophage stimulants, understanding the macrophage phenotype under label-free conditions is essential in the development of pharmacological agents designed to influence macrophage behavior.

MATERIALS AND METHODS

The murine macrophage cell line, J774A.1 was analyzed untreated (M0), stimulated with LPS to drive M1 polarization, stimulated with IL-4 to drive M2 polarization. For co-culture studies, SKOV-3 human ovarian adenocarcinoma cells were co-cultured with stimulated macrophages. Imaging data was obtained on a holographic time-lapse imaging cytometer HoloMonitor M4 (Phase Holographic Imaging, Lund, Sweden). The macrophages were tracked over a period of 24 hours by imaging at 5 min intervals and their motility patterns were assessed.

In this reports, three conditions are shown: unstimulated cells (Fig. 1A), LPS stimulated cells (Fig.1B), and IL4 stimulated cells (Fig. 1C). For each condition, a single frame is shown in Fig. 1 to present cell morphology and XY motion plots of the macrophages selected for long-term tracking. Time-lapse videos are made available to view cell morphology and changes in cell behaviors over a period of 5 hours.

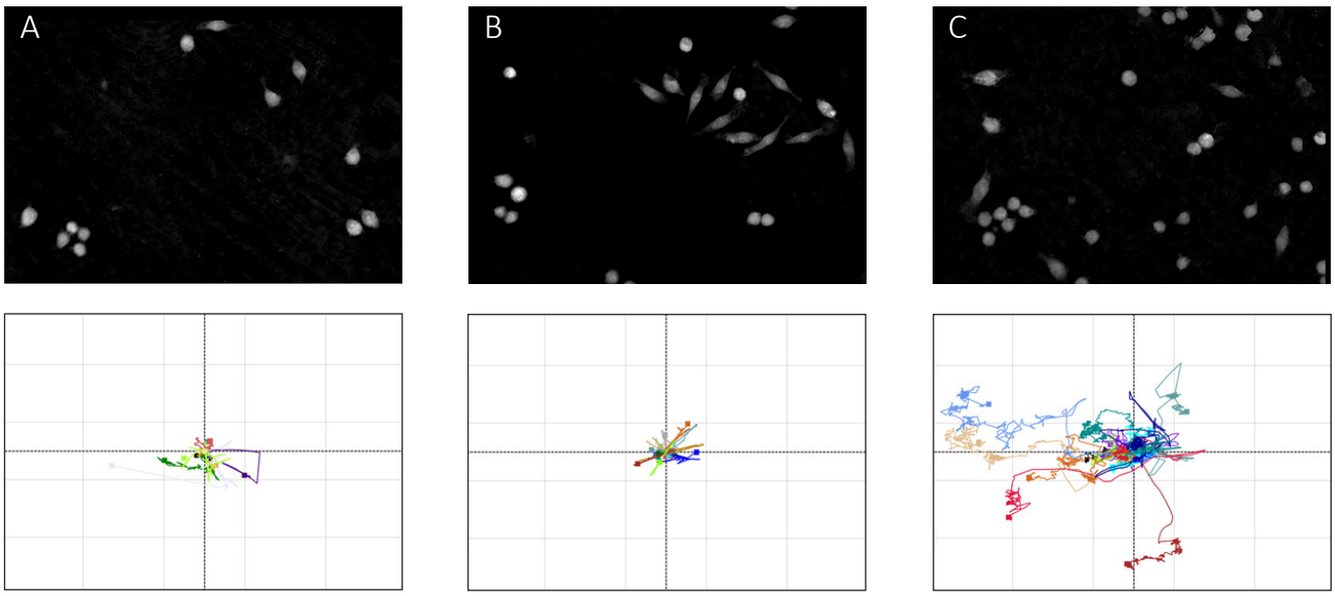

Figure 1. *Time-lapse holographic images of J774A.1 cells (A) untreated, (B) lipopolysaccharide stimulated for M1 phenotype and (C) interleukin-4 stimulated for M2 phenotype.*

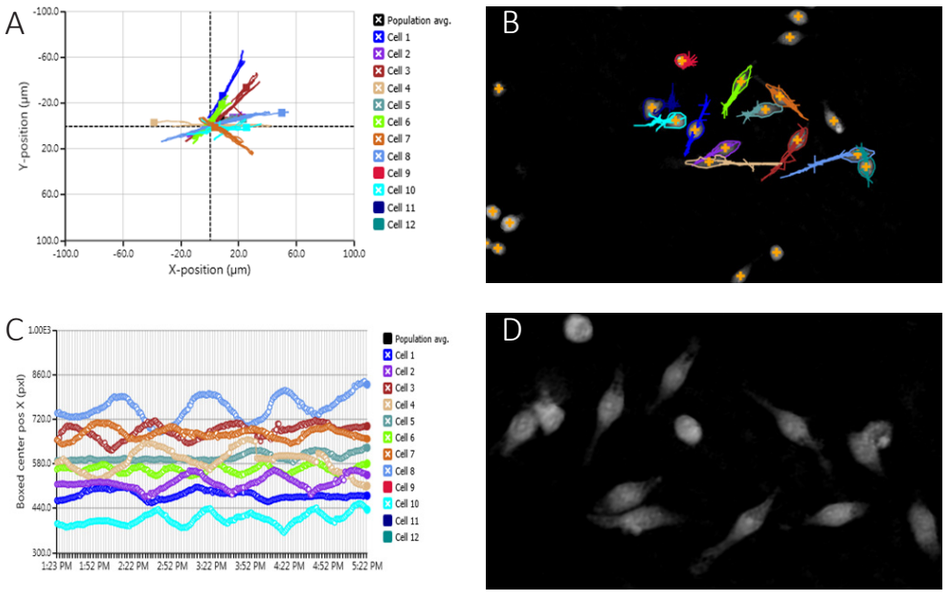

Figure 2. *Macrophage shuttling motion of LPS stimulated J774A1 cells (A) cell tracks centered in the graph, (B) cell tracks overlain on cells, (C) cell x-position plotted for 4 hours showing the sinusoidal motion, and (D) enlarged view of the cells.*

RESULTS AND DISCUSSIONS

In untreated cell cultures, we primarily see rounded cells and small amounts of cell movement, especially in cultures with low cell densities. While these cultures are unstimulated, and presumably in the M0 polarization state, in cultures with damaged cells, we see evidence of phagocytic absorption of small of cell debris and probable activation of some of the cells. These cells become more mobile.

In cultures stimulated with LPS, which is known to induce M1 polarization, the cell morphology of some of the cells changes to bisymmetrical and elongated. Large podosomes extended alternatively from either pole of the cell. The result is a characteristic shuttling or zig-zag motion, as seen in Fig. 2.

In cells stimulated with IL4, the cells maintained a rounded morphology, and initially move randomly in “starburst patterns”. As the cells move towards other cells in the culture – the movements become an open starburst pattern, with relatively large distances covered with many random changes in direction (note the red-orange track in Fig. 3).

CONCLUSIONS

The recently developed HoloMonitor M4 label-free kinetic imaging analysis platform provides a new tool for understanding the macrophage phenotype changes undergoing exposure to various stimulants. The results are not hindered by toxic stains and phototoxicity typical in confocal microscopy and other common types of single cell analysis. These preliminary studies synergize with the investigation of other factors affecting macrophage phenotype.

REFERENCES

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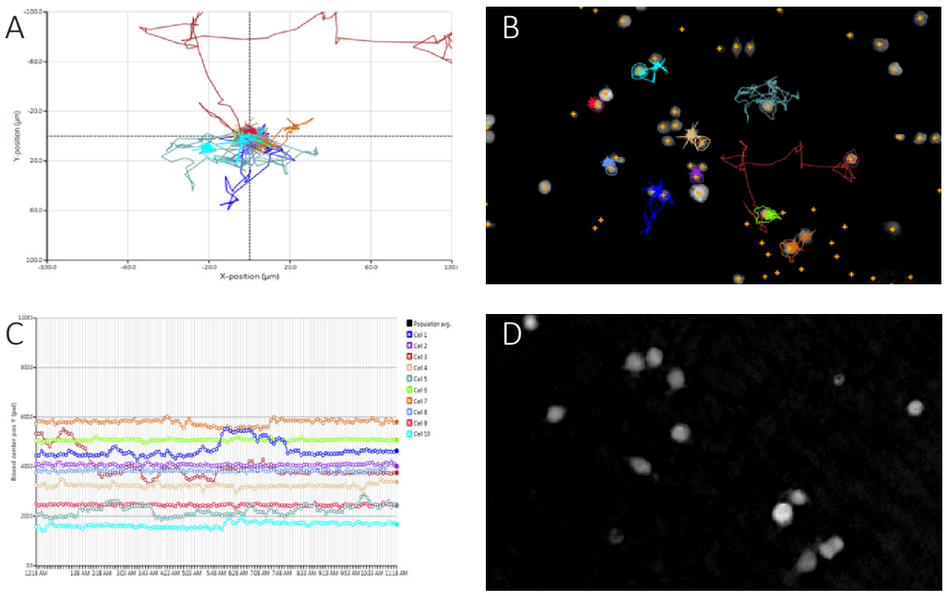

Figure 3. *Macrophage starburst motion of IL4 stimulated J774A1 cells (A) cell tracks centered in the graph, (B) cell tracks overlain on cells, (C) cell x-position plotted for 4 hours showing random motion, and (D) enlarged view of the cells.*