Application Note

on

Macrophage Polarization

Northeastern University Series

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"HoloMonitor M4 provides for real-time imaging of cellular interactions between tumor cells and macrophages in a co-culture."

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Holographic Imaging-Based Functional Assessment of Macrophage Polarization in Co-Culture with Tumor Cells

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ABSTRACT

Tumor-associated macrophage re-polarization from predominant M2 to M1 phenotype can be an effective approach to achieve anti-tumor immunity and therapeutic response. Holographic time-lapse imaging cytometer HoloMonitor® M4 (Phase Holographic Imaging, Sweden) was used to assess the efficacy of reprogramming M2 polarized macrophages to a M1 polarized state in a macrophage-tumor co-culture system. Also, the miR-155 polarized macrophages were seen to cause tumor cells morphological changes suggesting cell death after co-culture indicating potential anti-tumor activity.

INTRODUCTION

Macrophages are one of the important micro-environmental components of solid tumors, whose polarization to M2 phenotype aids in regulation of all steps of tumor pathogenesis[1]. Thus prevention of these pro-tumoral macrophage functions can be an important strategy to attain anti-tumor activity.

Out of many approaches available, the phenotypic reprogramming or repolarization to the anti-tumoral M1 state is a promising approach[2]. HoloMonitor M4 based imaging modality can be used to track unstained macrophages by monitoring their morphology and motility. In this study, we have explored the potential of genetic (microRNA mediated) phenotypic reprogramming of macrophages using non-viral water-in-oil-in-water (WOW) multiple emulsion-based intracellular delivery system.

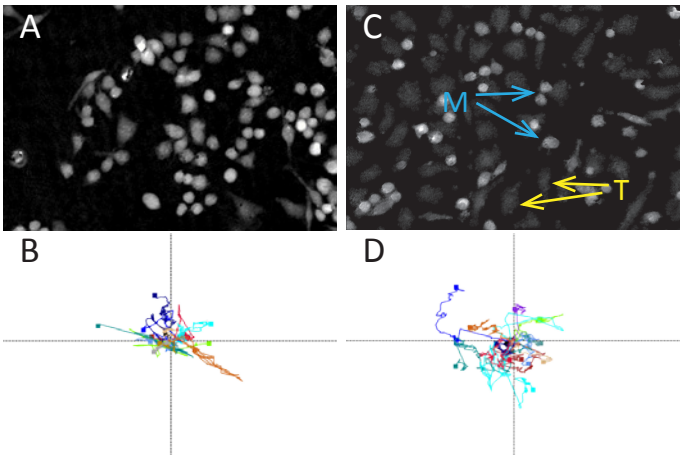


Figure 2. *MicroRNA-155 multiple emulsion treated macrophage culture, representative field of view (A) and plot of spatial movements (B). MicroRNA-155 multiple emulsion treated macrophage-SKOV-3 tumor cell co-culture, representative field of view (C) and plot of spatial movements (D).*

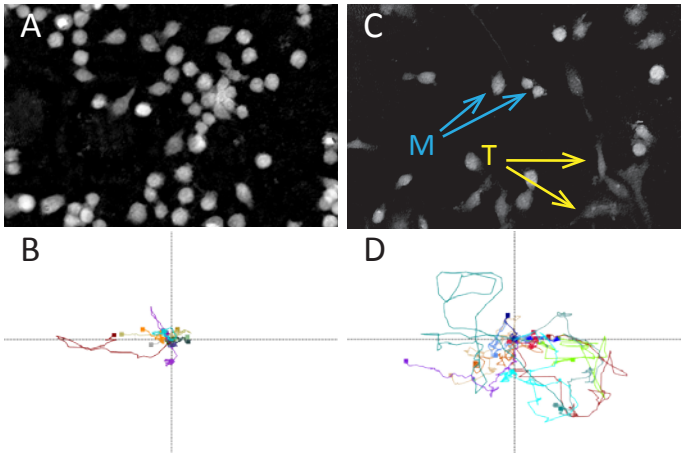


Figure 1. *Blank multiple emulsion treated macrophage culture, representative field of view (A) and plot of spatial movements (B). Blank multiple emulsion treated macrophage - SKOV-3 tumor cell co-culture, representative field of view (C) and plot of spatial movements (D). Arrows pointing at macrophages (M) and tumor cells (T).*

MATERIALS AND METHODS

J774A.1 murine macrophage cell line was purchased from American Type Culture Collection (ATCC; Manassas, VA) and grown using Dulbecco's Modified Eagle Medium (DMEM; Mediatech Inc., Manassas, VA) supplemented with 10% fetal bovine serum (FBS; Fisher Scientific, Pittsburg, PA) and 1% penicillin-streptomycin-amphotericin B antimicrobial combination (Pen/Strep/Amphotericin B; Lonza Walkersville Inc., Walkersville, MD) in a T-25 flask at 37˚ C and 5% CO_2. After overnight culture, the cells were treated with either blank multiple emulsion or multiple emulsion system encapsulating miR-155 encoding plasmid for 4 hours. Thereafter the cells were washed and incubated for 56 hours to allow for production of microRNA 155. On analysis day, the macrophage populations were first imaged for a period of 5 hours at 5 minute intervals to characterize cellular motility and morphology. Cultures of SKOV3 TR were prepared and imaged for 2 hours at 5 minute intervals to obtain base line tumor cell measurements. Thereafter either blank, multiple emulsion treated or miR-155 multiple emulsion polarized macrophages were added to set up a co-culture system. Imaging was performed using HoloMonitor M4 on this co-culture system for 16 hours at 5 minute time intervals to monitor the morphology and movements of the macrophages.

For each of the experimental cases (blank multiple emulsion treated and miR155 multiple emulsion treated macrophages, with and without SKOV-3 ovarian cancer cells in the culture) we present an individual image frame, as well as a video showing the movements of the cells. At the start of the analysis 10 to 12 visually identifiable macrophages in each experimental set were selected for tracking based on their brighter coloration than tumor cells. Their movements are displayed in spatial X-Y plots.

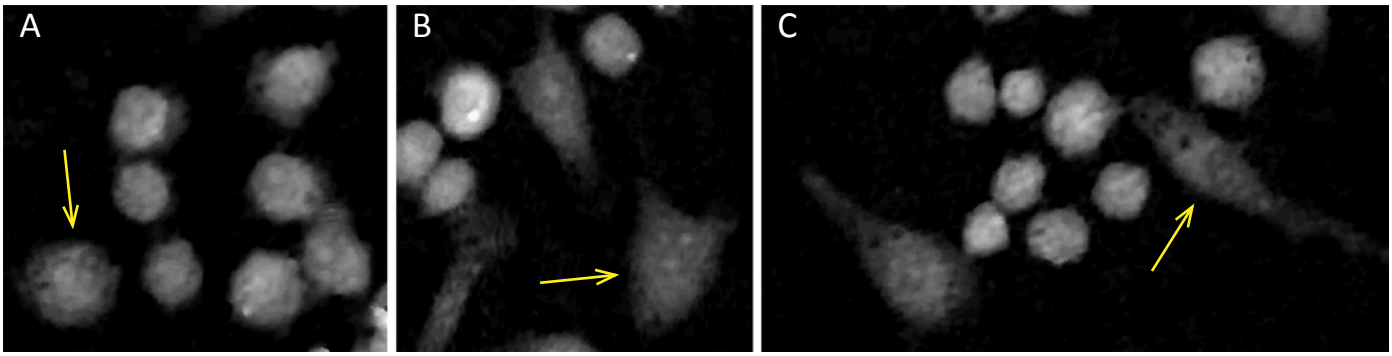


Figure 3. *Macrophage morphologies of MicroRNA-155 multiple emulsion treated macrophages (representative cells are marked with arrows): circular morphology (A); angular morphology (B); shuttling morphology (C).*

RESULTS AND DISCUSSIONS

In the blank multiple emulsion treated macrophage culture, the cells predominately present a rounded morphology (Fig. 1A), with only slight amounts of motion (Fig. 1B). A few of the macrophages exhibit shuttling behavior as was seen in LPS stimulated macrophages in other studies.

In the case of blank multiple emulsion treated macrophages co-cultured with the SKOV-3 cells, the macrophage cells predominately maintain a rounded morphology (Fig. 1C), but also exhibit high amounts of motility (Fig. 1D). Their movements are in an "open starburst" type pattern, with large distances covered with numerous random appearing changes in direction. The macrophages do not appear to be targeting the tumor cells.

The miRNA 155 multiple emulsion treated macrophage culture contains a mixture of macrophage morphologies, ranging from elongated "shuttle motion" types exhibiting large podosomes, to round "starburst motion types", and an intermediate morphology of an "angular phenotype", with three to five sharp corners (Fig. 2A and Fig. 3).

In the miRNA 155 multiple emulsion treated macrophage SKOV-3 co-culture (Fig. 2C), the macrophages are very active, and appear to be targeting the tumor cells. Interestingly, in the course of the videos, the tumor cells appear dimmer as the culture goes on, suggesting a loss in structural integrity, consistent with cytolytic hits from the macrophages. This observation was confirmed by immunofluorescence studies not shown here.

CONCLUSIONS

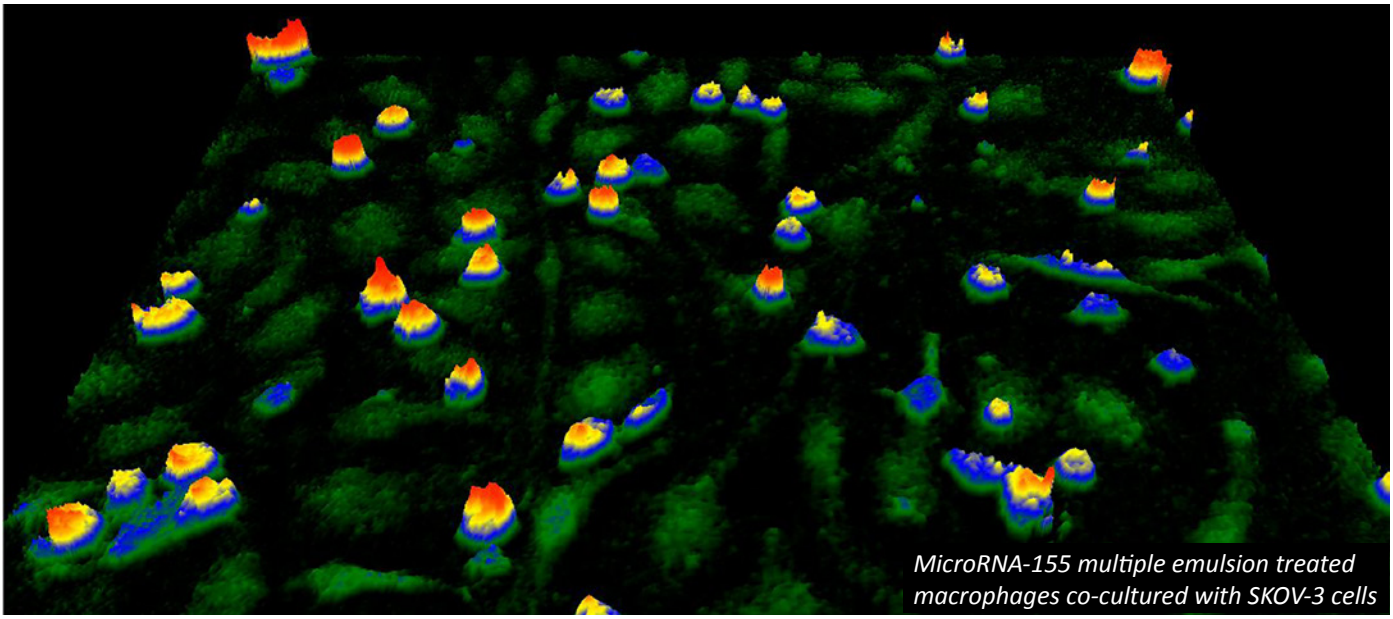
HoloMonitor M4 provides for real-time imaging of cellular interactions between tumor cells and macrophages in a co-culture system and thus can be used to investigate the effects of M1 polarized macrophages on cancer cells. Another advantage of this technique is the non-invasive nature that allows subsequent cellular staining for further analysis upon conclusion of holographic imaging. This includes but not limited to vital fluorescent DNA, permeability and CD11b image analysis. In this study, we found that the miRNA 155 appears to have been effective in re-polarizing the macrophages in the sample to the M1 polarization state, providing a potential new weapon in tumor eradication.

TIME-LAPSE VIDEO LINKS

- Blank multiple emulsion treated macrophage culture
- Blank multiple emulsion treated macrophages co-cultured with SKOV-3 tumor cells
- MicroRNA-155 multiple emulsion treated macrophage culture
- MicroRNA-155 multiple emulsion treated macrophage co-cultured with SKOV-3 cells

REFERENCES

1. D. M. Richards, J. Hettinger, and M. Feuerer, 'Monocytes and Macrophages in Cancer: Development and Functions', Cancer Microenviron, 6 (2013), 179-91.
2. X. Tang, C. Mo, Y. Wang, D. Wei, and H. Xiao, 'Anti-Tumour Strategies Aiming to Target Tumour-Associated Macrophages', Immunology, 138 (2013), 93-104.



MicroRNA-155 multiple emulsion treated macrophages co-cultured with SKOV-3 cells