Characterizing MDSC Migration After Functionalization with Targeted Microparticles

Rumya Raghavan, 2017, Biological Engineering

Advisor: Kavya Rakhra, Bioengineering

Principle Investigator: Darrell Irvine, Biological and Materials Science Engineering

Introduction:

Cancers are responsible for nearly 7.6 million deaths per year and unfortunately most leading chemotherapeutic treatments are highly toxic to healthy cells. In an attempt to combat this issue, controlled drug delivery systems are used in which drugs are transported to the site of the tumor, thereby limiting toxicity and dosage concentration (Srivasta et al. 2012). We propose to use myeloid derived suppressor cells (MDSCs) attached to polymer backpacks as a drug delivery vehicle to achieve targeted drug delivery to the tumor site.

MDSCs are found in bone marrow and are commonly known for suppression of the anti-tumor response and facilitation of tumor metastasis. Tumors overexpress colony stimulating factors such as GMCSF, MCSF, and IL-6 which in turn stimulate growth and development of MDSCs. In response to these chemokines, MDSCs migrate to the tumor site where they inhibit the anti-tumor immune response.

The aim of this investigation is to harness this tumor-specific tropism of MDSCs in order to deliver drugs directly to the tumor site. Microparticles are attached as drug-carrying backpacks onto MDSCs. The specific particles chosen for use in this investigation were layered polymers with a cell-adhesive region, a payload region, and a release region for efficient cellular attachment and drug release (Swiston et al. 2010). The end goal is to use MDSCs to transport the drug loaded backpacks to the site of the tumor and unload the therapeutic agent.

Methods and Results:

CD11b+/Gr1+ MDSCs were isolated from spleens of tumor-bearing mice using magnetic-activated cell sorting (MACS) and the purity of the isolated MDSC population was evaluated using flow cytometry. MDSCs were stained with anti-CD11b FITC and anti-GR1 PE antibodies and analyzed. Flow cytometry results showed that 99.4% of the MDSCs expressed both CD11b and Gr-1 (Figure 2). These MDSCs then were conjugated to rhodamine labeled polymer backpacks (Figure 1) and were observed through the use of fluorescnce microscopy (Figure 3). MDSCs functionalized with backpacks were then run through a transwell polycarbonate membrane either toward control medium (IMDM) or tumor conditioned medium (TCCM) and the resultant migration was compared to control non-functionalized MDSCs. After the migration, the MDSCs that migrated through the transwell membrane were again observed under the fluorescent microscope. There were fewer MDSCs with attached backpacks as observed by the decreased red fluorescence observed post-migration (Figure 4). The migrated cells were then counted and these values were compared to the known amount of cells originally plated for the migration assay to calculate a percent migration. There was a significant difference between the percent of MDSCs with and without backpacks in TCCM and there was a large significant difference between the percent of migrated MDSCs in control versus tumor conditioned media in both MDSCs only and MDSCs with backpacks (Figure 5).

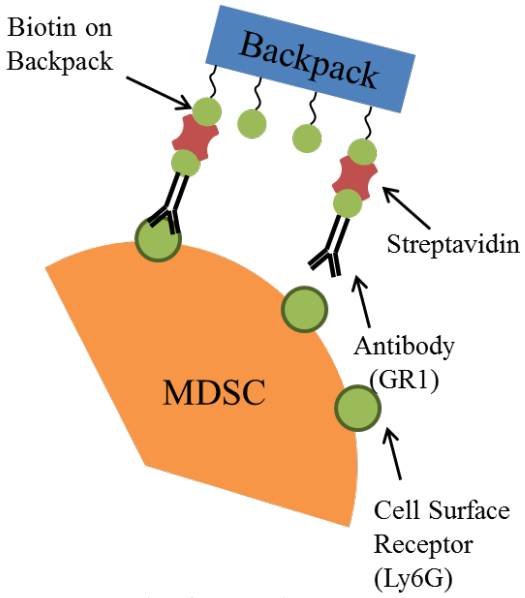
Conclusions and Future Direction:

The results from the migration assay demonstrate that the tumor conditioned media performed far better than the control media (IMDM) in terms of attracting MDSC migration. However, there was a significant decrease in migration (~30%) in migration towards TCCM when

backpacks were attached to MDSCs. This indicates that the backpacks are in fact impeding the motility of the MDSCs and affect tropism towards growth factors released by tumor cells. This could be caused by the backpacks either blocking chemical receptors on the MDSC surface or by the increased size of the MDSC-backpack complex. Future studies will investigate mechanisms through which migration and cellular viability can be improved both in-vitro and in-vivo.

Figures:

Figure 1: Backpack /MDSC Conjugation reaction occurs via an antibody/receptor interaction on MDSC surface



Antibody Attachment:
Biotinylated Backpacks Attach to Cells Using A Streptavidin Bridge and Biotinylated Antibody

Figure 2: FACS results showing that the isolated MDSCs were positive for surface markers GR1 (PE) and CD11b (FIT)

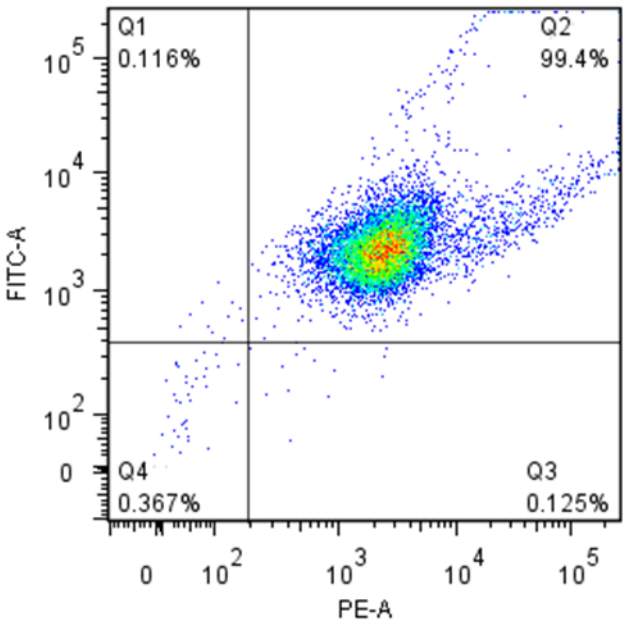


Figure 3: Florescence microscopy showing that the backpacks (labeled in red with rhodamine) successfully attached to the MDSCs

Figure 4: Florescence microscopy showing that fewer functionalized MDSCs migrated than were initially present.

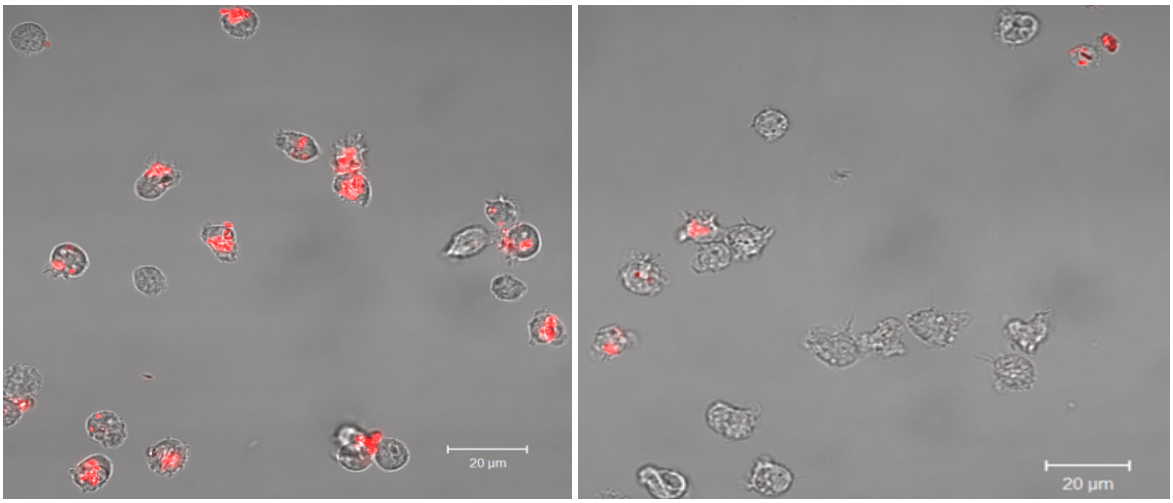
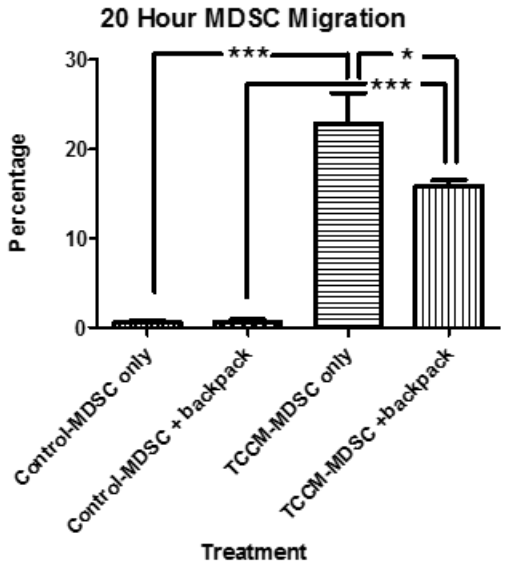


Figure 5: Percentage of migrated MDSCs toward control vs. TCCM media and with and without backpacks.



References:

Sinha, P., Okoro, C., Foell, D., Freeze, H., Ostrand-Rosenberg, S., & Srikrishna, G. (). Proinflammatory S100 Proteins Regulate the Accumulation of Myeloid-Derived Suppressor Cells. *The Journal of Immunology*, *181*, 4666-4675.

Srivasta, M., Zhu, L., Harris-White, M., Kar, U., Huang, M., Johnson, M., Lee, J., Elashoff, D., Strieter, R., Dubinett, S., Sharma, S. (). Myeloid Suppressor Cell Depletion Augments Antitumor Activity in Lung Cancer. *PLoS ONE, 7, E40677*

Swiston, A., Gilbert, J., Irvine, D., Cohen, R., Rubner, M. F. (). Freely Suspended Cellular "Backpacks" Lead to Cell Aggregate Self-Assembly. *Biomacromolecules*, *11*, 1826-1832.

Acknowledgements

I'd like to thank Darrell Irvine for allowing me to work in his lab, the Koch Institute for their wonderful facilities and openness to allowing undergraduate research and most importantly, my mentor Kavya Rakhra for being an incredible teacher and a devoted researcher. This work was conducted under Dr. Rakhra in conjunction with Rosanna Lim from the Rubner lab.