Modulation of Macrophage Polarization by Urolithin A

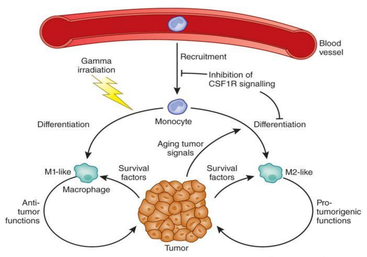
Alexandra M. Wright, Anne E. Geller, M.S., Xiaoling Hu, Dr. Jun Yan M.D., Ph.D.
Department of Medicine, James Graham Brown Cancer Center, University of Louisville

ABSTRACT

Tumor associated macrophages play a large role in promoting disease progression and have been identified as valid targets for cancer therapeutics. Reprogramming macrophages from an immunosuppressive phenotype (M2) to an immunostimulatory one (M1) presents a possible immunotherapeutic approach that may be modulated by products of the human microbiota. Considering this, we investigated the effect of Urolithin A (Uro-A), an intestinal microbial metabolite, on macrophage phenotype. Micromolar Uro-A treatment was examined in RAW 264.7 and J774 macrophage cell lines and bone marrow derived macrophages (BMDMs) and was quantified using real time PCR and flow cytometry. This study was conducted with the expectation that the presence of Uro-A would polarize the pro-tumorigenic M2 phenotype towards a more M1-like phenotype. Our data show that in specific conditions, macrophages polarized to the M2 phenotype can express fewer M2 surface markers and more M1 surface markers following treatment with Uro-A. These findings point to Uro-A as a potential modulator in the reversal of macrophages from the M2 to M1 state. Further, these findings may have implications in the use of Uro-A in cancer settings.

INTRODUCTION

- Undifferentiated macrophages derived from monocyte precursors (M0) may polarize to a variety of phenotypes depending on the conditions of the local tissue environment [2].

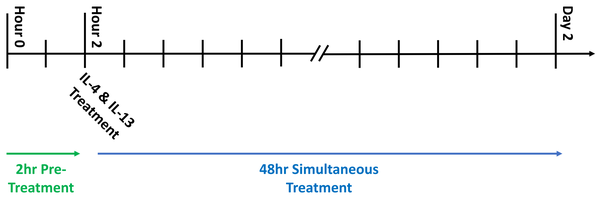


Reference: 1

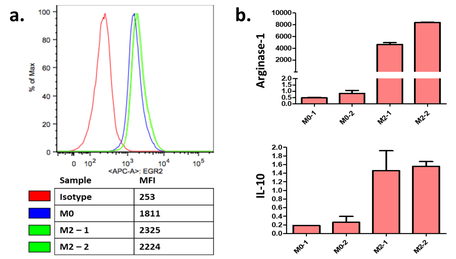
- M0 macrophages are classically activated to an M1, immunostimulatory phenotype in the presence of LPS IFN-gamma, or GM-CSF [2].
- M0 macrophages are alternatively activated to an M2, immunosuppressive phenotype in the presence of IL-4, IL-13, IL-10, or TGF-beta [2].
- Tumor-associated macrophages (TAMs) resemble the M2 phenotype and contribute to proliferation, invasion, and metastasis of tumor cells [3].
- In a clinical setting, increased TAM density in cancer patients is associated with decreased rates of relapse-free survival and overall survival [3].
- Uro-A, a major metabolite of ellagitannins in the intestine, has been shown to polarize the M1 phenotype towards an M2 phenotype in an inflammatory setting, indicating that Uro-A is involved in overall macrophage polarization [4].
- The anti-metastatic effects of Uro-A in colorectal cancer cells [5] and its anti-proliferative effects in liver [6] and prostate [7] cancer cells have been previously demonstrated.

METHODS

Urolithin A 48 hour Simultaneous Treatment vs. 2 hour Pre-Treatment:



RESULTS



Sample	MFI
Isotype	253
M0	1811
M2 – 1	2325
M2 – 2	2224

Figure 1: (a) Histogram produced by flow cytometric analysis on RAW 264.7 macrophages. M0 cells were grown in plain media, while M2 cells received 20 ng/ml IL-4 and IL-13 treatment. **(b)** Fold changes in expression of Arginase-1 and IL-10 as revealed by real time PCR.

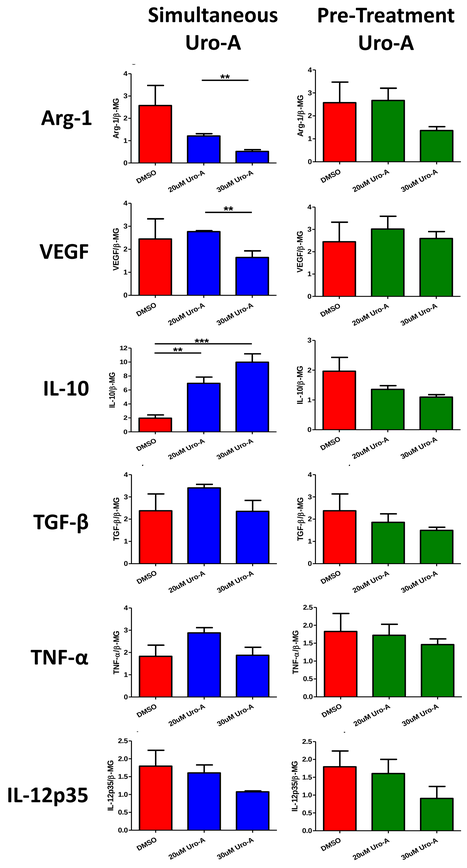


Figure 2: Real time PCR data on RAW 264.7 macrophages receiving the simultaneous Uro-A treatment (left) and receiving the Uro-A pre-treatment (right). Expressions of various M1 surface markers were quantified for cells treated with DMSO only, 20µmol Uro-A, and 30µmol Uro-A.

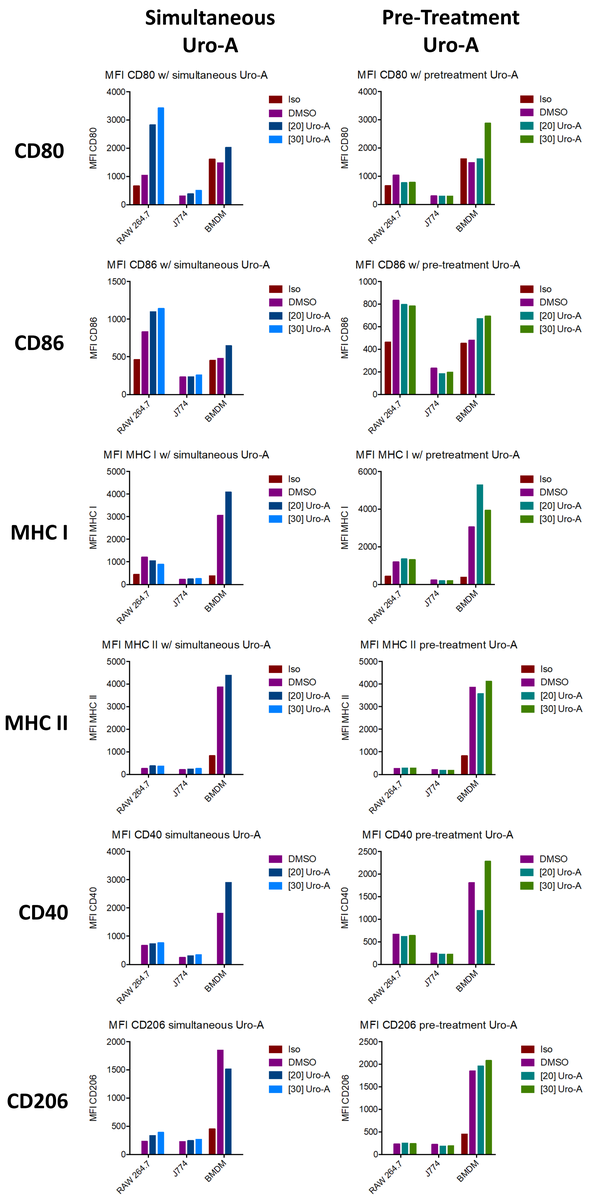


Figure 3: Flow cytometry data for RAW 264.7 macrophages receiving the simultaneous Uro-A treatment (left) and receiving the Uro-A pretreatment (right). Expressions of several M2 surface markers in isotypes and cells treated with DMSO only, 20µmol Uro-A, and 30µmol Uro-A.

CONCLUSIONS

- RAW 264.7 macrophages grown in media treated with 20 ng/ml IL-4 and IL-13 displayed an enhanced upregulation of M2 markers compared to those cells grown in plain media.
- Increasing concentrations of Uro-A treatment resulted in decreased expression of various M2 markers in macrophages polarized to M2. Additionally, increased Uro-A concentrations resulted in the upregulation of several M1 phenotypic markers.
- As compared to the 2 hour pre-treatment with Uro-A, a 48 hour simultaneous treatment of Uro-A with IL-4 and IL-13 resulted in more significant expression of surface markers on RAW 264.7 and J774 cells as well as on primary BMDMs.

FUTURE DIRECTIONS

- In order to more clearly define the relationship between the concentration of Uro-A and expression of phenotypic macrophage markers, this study will be repeated with a titration of Uro-A treatments at higher concentrations, specifically at 40µmol and 50 µmol.
- Further M2-polarization studies could reveal additional information about the relationship between Uro-A and IL-10 and whether IL-10 can be considered a reliable marker of the M2 phenotype.
- Potentially, if future research on Uro-A supports its role as an immuno-stimulant, immunomodulation by Urolithin A could be implemented in patients through dietary consumption of probiotics or fecal microbiota transplantation with the purpose of inducing enhanced immune responses to cancer.

REFERENCES

1. Bronte, V., & Murray, P. J. (2015, February 05). Understanding Local Macrophage Phenotypes In Disease: Modulating macrophage function to treat cancer.
2. Bio-Rad. Macrophage Polarization Mini-review.
3. Chanmee, T., Ontong, P., Konno, K., & Itano, N. (2014, September). Tumor-Associated Macrophages as Major Players in the Tumor Microenvironment.
4. Boakye, Y. D., Groyer, L., & Heiss, E. H. (2018, January). An increased autophagic flux contributes to the anti-inflammatory potential of urolithin A in macrophages.
5. Zhao, W., Shi, F., Guo, Z., Zhao, J., Song, X., & Yang, H. (2018, February). Metabolite of ellagitannins, urolithin A induces autophagy and inhibits metastasis in human sw620 colorectal cancer cells.
6. Wang, Y., Qiu, Z., Zhou, B., Liu, C., Ruan, J., Yan, Q., . . . Zhu, F. (2015, August). In vitro antiproliferative and antioxidant effects of urolithin A, the colonic metabolite of ellagic acid, on hepatocellular carcinomas HepG2 cells.
7. Vincinata, R., Zhang, Y., Henning, S. M., Li, Z., & Heber, D. (n.d.). Urolithin A and Ellagic Acid Inhibit Prostate Cancer Through Different Molecular Mechanisms: Implications of Gut Microbiome Metabolism for Cancer Prevention.

ACKNOWLEDGEMENTS

This research was supported by the R25-CA134283 grant from the National Cancer Institute through the University of Louisville Cancer Education Program.