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Urea suppresses the alternative activation of murine macrophages

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Objectives: Even though widely known to be harmless, urea has been reported to impede the intracellular transport of arginine in vascular endothelial cells and attenuate glycolysis in pancreatic beta cells. In this study, we investigated whether high levels of extracellular urea affect macrophage functions.

Methods: Peritoneal macrophages and bone marrow-derived macrophages (BMDMs) were treated with LPS and IL-4 for M1 and M2 polarization, respectively, in the presence of various concentrations of urea. Expression of arginase-1 and iNOS was examined by flow cytometry. Expression of M2 marker genes were also analyzed by qPCR. Seahorse real-time cell metabolic analysis was performed to check oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of IL-4-treated BMDMs.

Results: We found that physiologically high concentrations of urea attenuate the IL-4-induced upregulation of arginase-1 in murine macrophages in a dose-dependent manner at both mRNA and protein levels. Expression of other genes that are related to alternative activation of macrophages, such as Fizz1 and Ym1, was also significantly reduced by urea. On the other hand, urea had little effect on LPS-induced M1 polarization. Transcriptomic analysis of M2-polarized BMDMs by RNA sequencing revealed that urea tends to down-regulate the gene sets associated with mTOR signaling, glycolysis, and oxidative phosphorylation. In line with these results, phosphorylation of S6 protein and AKT, which are downstream targets of mTORC1 and mTORC2, respectively, was decreased by urea treatment. ECAR and OCR were also decreased in urea-treated M2 BMDMs. The urea-induced attenuation of M2 polarization was not obvious in the presence of Torin, a pan mTOR inhibitor, suggesting that the inhibitory effect of urea is mediated by downregulation of mTOR signaling.

Conclusions: Based on these results, we propose that, contrary to the traditional knowledge assuming urea as an inert molecule, urea suppresses the M2 polarization of murine macrophages, likely via attenuation of mTOR signaling.

Figure 1. The M2 polarization of macrophage is attenuated by urea

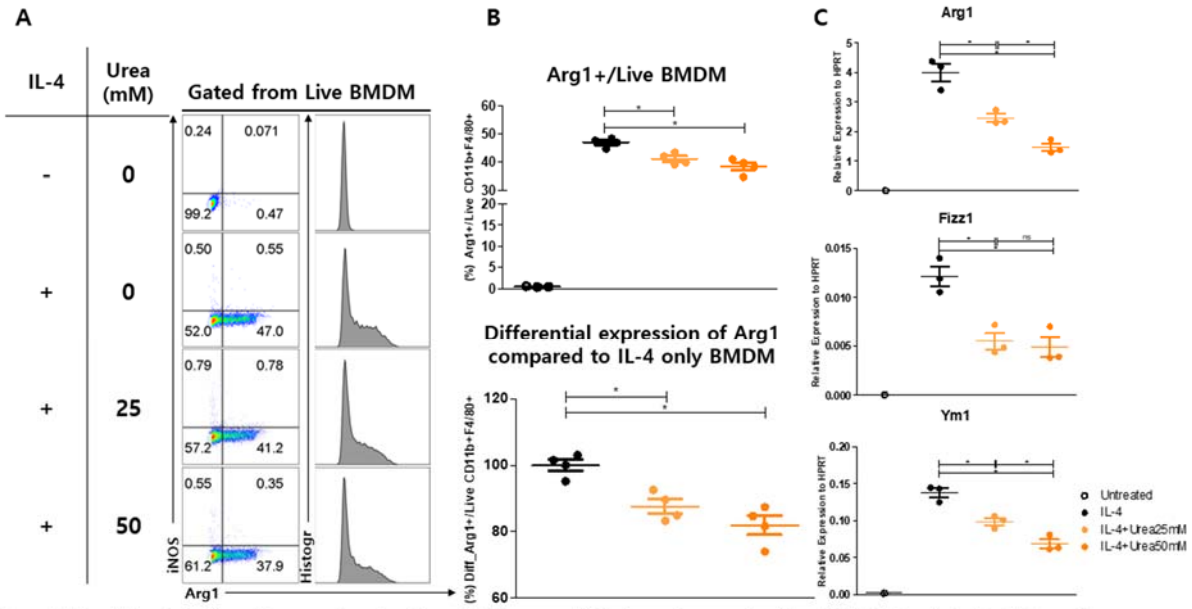

Figure 1. The M2 polarization of macrophage is attenuated by urea. (A) Arginase 1 expression from BMDM after IL-4 stimulation with or without urea (B) Differential expression of arginase 1 compared to IL-4 stimulated BMDM. (C) qPCR for M2 marker genes from IL-4 stimulated BMDM with or without urea. *P<0.05, **P<0.01 (Mann-Whitney U test)

Figure 2. Bulk RNA seq from M2 BMDM

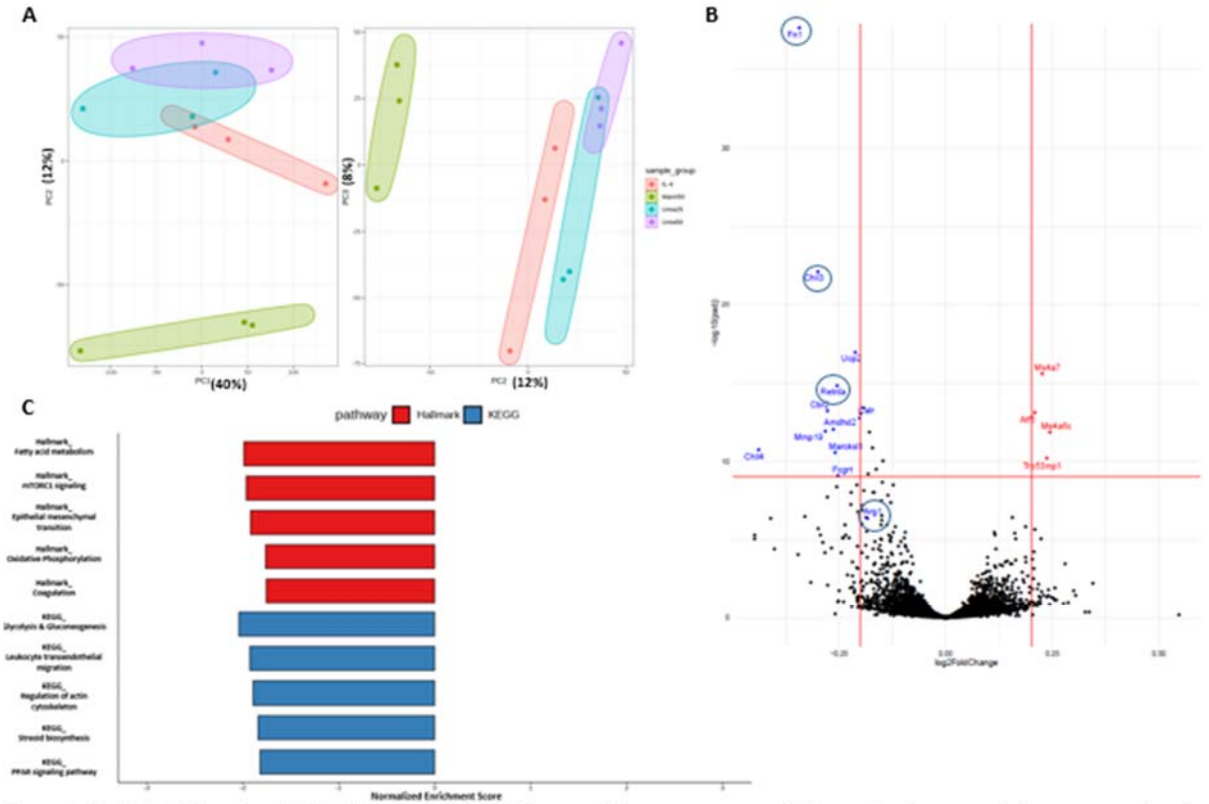

Figure 2. The Bulk RNA seq from M2 BMDM showed that high extracellular urea attenuated M2 associated genes and the genes associated with mTOR pathway. (A) PCA plot from BMDM treated with IL-4 (Red), IL-4+Urea 25mM (Blue), IL-4+Urea50mM (purple) and IL-4+Mannitol 50mM (green) (B) Volcano plot shows upregulated and downregulated genes in IL-4 treated macrophage with urea. M2 marker genes are indicated by blue circle. (C) Bar plot for enriched gene sets from Hall mark gene set and KEGG pathway gene set.