Secreted factors from M1 macrophages drive prostate cancer stem cell plasticity by upregulating NANOG, SOX2, and CD44 through NFκB-signaling

Kainulainen, K[1], Niskanen, E.A[1], Kinnunen, J [1], Mäki-Mantila, K[1], Hartikainen, K[1], Paakinaho, V[1], Malinen, M[2], Pasonen-Seppänen S[1]* and Ketola, K[1]*

[1]Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio, Finland
[2]Department of Forestry and Environmental Engineering, South-Eastern Finland University of Applied Sciences, Kouvola, Finland

Background. The inflammatory tumor microenvironment (TME) is a key driver for tumor-promoting processes. Tumor-associated macrophages are one of the main immune cell types in the TME and their increased density is related to poor prognosis in prostate cancer. Since M1 macrophages are primarily known to have antitumor effects, reprogramming of pro-inflammatory macrophages is suggested as cancer immunotherapy in several cancer types including prostate cancer. However, the overall impact of pro-inflammatory macrophages on tumor progression is still unclear.

Methods. We investigated the influence of pro-inflammatory (M1) and immunosuppressive (M2) macrophages on prostate cancer lineage plasticity. THP-1 cells were differentiated and polarized to M0, M1, and M2 macrophages, macrophage culture media were collected and the conditioned medium was administered to LNCaP and C42B cells. The effects of secreted factors on prostate cancer cells were studied using AR activation assays, RNA-sequencing, qPCR, western blotting, proliferation and apoptosis assays, immunofluorescence stainings and microscopy. In addition, the association of CSC plasticity markers with M1 macrophage infiltration in prostate cancer adenocarcinoma patients was estimated using TIMER2.0 database.

Results. Our findings reveal that M1 macrophage secreted factors upregulate genes related to stemness while downregulating genes associated with androgen response in prostate cancer cells. The expression of cancer stem cell (CSC) plasticity markers NANOG, KLF4, SOX2, OCT4, and CD44 was stimulated by the secreted factors from M1 macrophages. Moreover, AR and KLK3 were suppressed in LNCaP cells treated with secreted factors from M1 macrophages. Inhibition of NFκB signaling using an IKK16 inhibitor resulted in downregulation of M1 macrophage -induced NANOG, SOX2, and CD44 and CSC plasticity. Furthermore, the TIMER2.0 demonstrated a significant positive correlation of KLF4, SOX2, and CD44 expression with M1 macrophage infiltration in prostate adenocarcinoma.

Conclusions. Our study highlights that the secreted factors from M1 macrophages drive prostate cancer cell plasticity by upregulating the expression of CSC plasticity markers through NFκB signaling pathway. The results in prostate adenocarcinoma also suggest that the expression of stem cell plasticity genes correlate with TME infiltration of M1 macrophages. Moreover, we found that factors secreted from M1 macrophages suppress AR signaling. These outcomes indicate that inflammatory TME has a role in tumor progression and pro-inflammatory macrophages highly influence prostate cancer cell transcriptome. Our findings suggest that M1 macrophages play a role in tumor promotion, which should be taken into account when considering reprogramming therapy.

Funding. The work was supported by the Research Council of Finland, Cancer Foundation Finland, Sigrid Jusélius foundation, Finnish Cultural foundation, Finnish Cultural foundation North Savo Regional Fund, the Cancer Society of North Savo, Kuopio University foundation and Paavo Koistinen foundation.

We have no conflicts of interest to disclose.