Macrophage re-education in prostate cancer: subversion of inflammatory macrophages to a mixed immunosuppressive tumor associated-macrophage phenotype

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Background
Tumor-associated macrophages (TAMs) are known to influence the tumor microenvironment (TME) supporting tumor progression. TAM are versatile cells implicated in different immune functions influenced by local factors, whether immune-stimulatory or immunosuppressive. Many questions remain as to the origin, development, and function of TAMs within the prostate TME. To understand the origin of this population, we analyzed phenotypical and functional aspects of human macrophages using analysis of radical prostatectomy specimens and *in vitro* co-culture models of macrophages and prostate cancer cells.

Methods
Tumor infiltration by the immunosuppressive M2 CD163+ TAMs was analyzed by immunohistochemistry (IHC) in a cohort of 98 patients with locally advanced PCa and long clinical follow-up. Biopsies taken in tumoral and non-tumoral zones of surgically removed prostates (radical prostatectomy specimens) were cultured *ex vivo* for 72 h, followed by cellular dissociation and flow cytometry analyses for a panel of macrophage markers. Human peripheral blood mononuclear cells were used to derive inflammatory M1 or immunosuppressive M2 macrophages which were co-culture with human prostate cancer cells to evaluate the effect of tumor cells on the macrophage phenotype.

Results
IHC studies identified CD163+ macrophages in tumor-adjacent normal epithelium, but not in tumoral regions, as a significant predictor of the development of metastases or PCa death. Flow cytometry analyses of radical prostatectomy specimens identified TAMs as frequently expressing both pro-inflammatory M1 (CCR7+) and immunosuppressive M2 (CD163+) markers. We show that prostate cancer cells subvert M1 macrophages into M2 macrophages by loss of function and up-regulation of CD163 marker. Further, we observed that the milieu-induced transition between immunosuppressive M2 to pro-inflammatory M1 macrophages is abrogated by the presence of PCa cells. Using RNA sequencing, we show that human macrophages subverted by PCa cells show alterations in the chemokine network which may recapitulate TAMs characteristics.

Conclusion
Together, our results suggest that prostate TAMs originate from infiltrating inflammatory M1 macrophages which are subsequently reprogrammed by PCa cells and the cytokine milieu. We also demonstrate that PCa cells influence TAM phenotype more significantly than the cytokine milieu.