Key Factors Released by Tumor-Associated Macrophages Drive Cancer Cell Invasiveness via IL 6/STAT3 Signaling

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Introduction

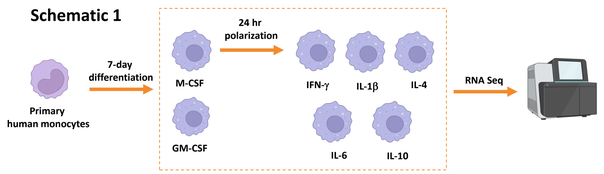
Tumor-associated macrophages (TAMs) are critical drivers of tumor progression and metastatic dissemination through their ability to shape the tumor microenvironment. Distinct macrophage subsets display diverse transcriptional and secretory profiles, yet the key mediators of cancer cell invasiveness remain incompletely defined. In parallel, stromal components such as fibroblasts—particularly cancer-associated fibroblasts (CAFs)—play an essential role in modulating tumor behavior. Here, we combine a human macrophage platform, transcriptomic data reuse, and physiologically relevant 3D tumor models to identify macrophage-derived drivers of invasion within a multicellular context.

Methods

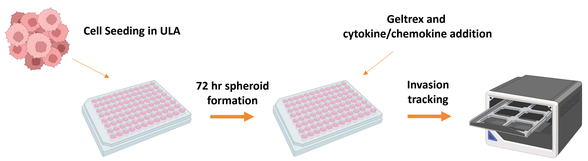
Primary human monocytes were differentiated and polarized into defined macrophage subsets representing TAM-like states (Schematic 1). Transcriptomic datasets were analyzed to identify differentially expressed cytokines, extracellular matrix (ECM)-modulating genes, and pro-invasive signaling pathways, enabling prioritization of candidate mediators.

Cancer cell lines spanning a range of invasive potential were incorporated into 3D co-culture models with fibroblasts, capturing key aspects of the tumor microenvironment. Fibroblasts were included as active stromal components, with potential conditioning toward CAF-like phenotypes in the presence of cancer cells. Invasion assays were performed using biologically relevant ECM system Geltrex™. Cells were treated with recombinant IL-6 and CXCL8, and STAT3 pathway inhibitor. Invasion dynamics were monitored in real time using IncuCyte® live-cell imaging, enabling quantitative, kinetic assessment of cell invasion (Schematic 2).

Schematic 1

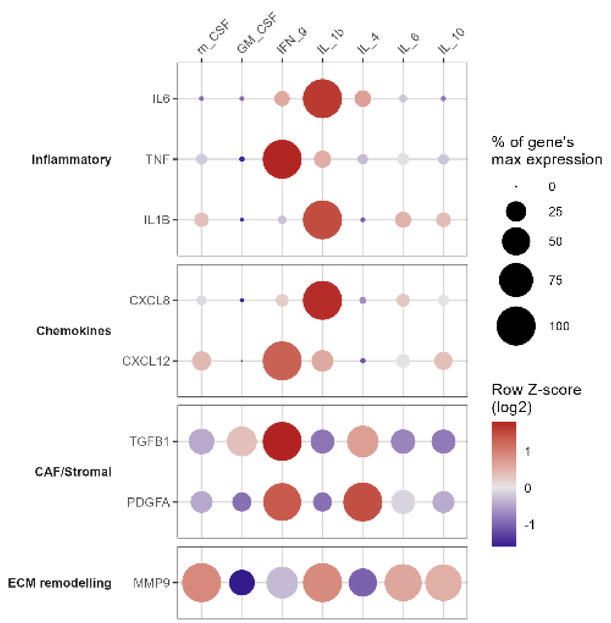


Schematic 2



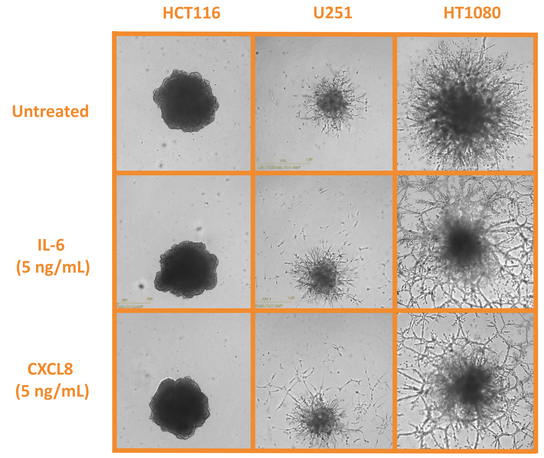
Dynamic Regulation of Invasion-Associated Genes Across *In Vitro*–Generated TAM Subtypes

Figure 1. Transcriptomic analysis of seven distinct *in vitro*–generated TAM subtypes was performed to investigate the regulation of key invasion-associated genes. Bubble plot showing RNA-seq gene expression across macrophage differentiation conditions (n = 3 donors per condition). Genes (rows) are grouped by functional category (inflammatory, chemokines, CAF/stromal, and ECM remodeling), while columns represent macrophage differentiation conditions. Bubble size indicates mean transcript abundance (TPM) across donors, expressed relative to the highest mean TPM observed for that gene across all conditions (100% = highest expression for that gene). Bubble color represents the row Z-score of $\log_2$(mean TPM + 1) calculated across conditions for each gene, ranging from low (blue) to high (red) relative expression. Both bubble size and color are scaled independently for each gene; therefore, neither Z-scores nor bubble sizes should be compared between genes, but only across conditions within the same gene.



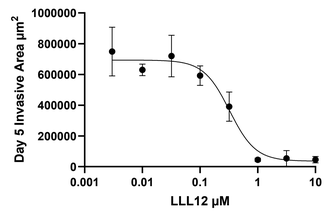
Differential Basal Invasiveness and Responsiveness to IL-6 and CXCL8 Across Cancer Cell Types

Figure 2. HCT116, U251 and HT1080 cancer cells exhibit distinct invasive capacities. Representative images acquired using the IncuCyte® live-cell imaging system illustrate the presence or absence of invasive behavior across the different cell lines.



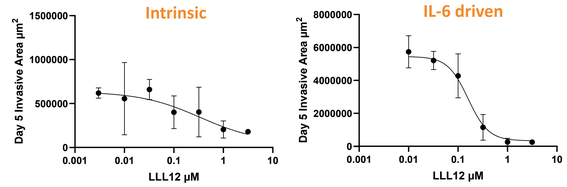
Invasive U251 Cells Are Unresponsive to Exogenous IL-6 and IL-8 but Show Reduced Invasion Following STAT3 Blockade

Figure 3. STAT3 inhibition with LLL12 suppresses the intrinsic invasive capacity of U251 cells in a dose-dependent manner. The graph shows the mean invasive area ± S.E.M. from ≥3 independent wells.



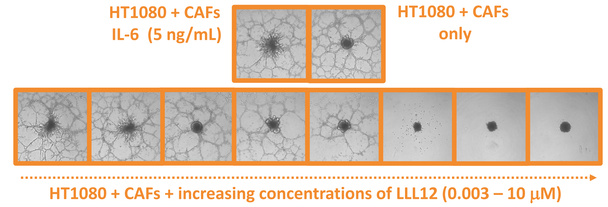
STAT3 Inhibition Suppresses Both Basal and IL-6/CXCL8-Induced Invasion in HT1080 Cells

Figure 4. STAT3 inhibition with LLL12 suppresses both the intrinsic and IL-6-driven invasive capacity of HT1080 cells in a dose-dependent manner. The graph shows the mean invasive area ± S.E.M. from ≥3 independent wells.



CAFs Promote HT1080 Invasion Through a STAT3-Dependent Mechanism

Figure 5. CAF incorporation increased HT1080 spheroid invasion, which was not further enhanced by exogenous IL-6. Representative IncuCyte® images show dose-dependent inhibition of CAF-driven invasion by the STAT3 inhibitor LLL12.



Conclusions

1. **Human-Relevant Macrophage Platform:** Primary human macrophage models capture dynamic invasion-associated biology and enable assessment of compounds that modulate macrophage-driven tumor invasion.
2. **Multi-Cellular Invasion Solutions**: Flexible assay formats incorporating macrophages, conditioned media, tumor cells, and fibroblasts allow investigation of both tumor-intrinsic and microenvironment-driven invasion mechanisms.
3. **Scalable Drug Discovery Screening:** Robust 96-well plate assays support efficient screening and ranking of therapeutic candidates targeting cancer invasion.
4. **Mechanistic Insights Beyond Invasion:** Platforms can be integrated with immunofluorescence imaging, cytokine/chemokine profiling, and gene expression analysis to provide deeper understanding of compound mode of action.

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