142. Hyal2/Hyal1-expressing Myeloid Cells as a target

(University of Florida)

5TH KDDF GLOBAL C&D TECH FAIR

Asset Overview

Product Type	Antibody
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	Tumor-associated myeloid cells
MoA	Increased fragmentation of extracellular HA and accumulation of low molecular weight HA (LMW-HA) in tumor tissue was associated with elevated production of multiple inflammatory cytokines, chemokines, and angiogenic factors.
Brief Description	• These antibodies can diagnose cancer or improve cancer immunotherapy by targeting a population of tumor-specific myeloid cells that contribute to tumor progression. Within the tumor microenvironment, certain cell types can cause immunosuppression, which promotes tumor progression and worsens prognosis. • Identifying these cell types can better inform cancer diagnosis and targeting them can improve the immune response during cancer therapy. Cancer diagnosis often requires invasive medical procedures difficult for some patients, such as biopsies or endoscopies, and common diagnostic procedures lack precision to track tumor response or recurrence after treatment. Furthermore, while immunotherapy holds great promise for cancer treatment, the majority of patients do not respond to available immunotherapies, which often fail to overcome immunosuppression. • Researchers at the University of Florida have identified a subset of myeloid cells that express hyaluronidase to promote immunosuppression within the tumor microenvironment. An antibody targeting hyaluronidase-expressing cells can detect these tumor-specific cells and eliminate them for immunotherapy.
Intellectual Property	WO2021252519A1
Publication	Hyal2 Expression in Tumor-Associated Myeloid Cells Mediates Cancer-Related Inflammation in Bladder Cancer. Tumor Biology and Immunology, (2021)
Inventors	Sergei Alekseyevich KUSMARTSEV, Paul CRISPEN, Paul R. DOMINGUEZ-GUTIERREZ

Highlights

- Requires a blood sample only, allowing minimally invasive cancer diagnosis
- Monitors efficacy of other therapies, precisely tracking tumor response and recurrence
- Targets a cell population that causes immunosuppression within the tumor microenvironment, helping turn a "cold" tumor to "hot"
- Antibodies administer directly or inform development of CAR-T cells that also target Hyal2/Hyal1-expressing cells

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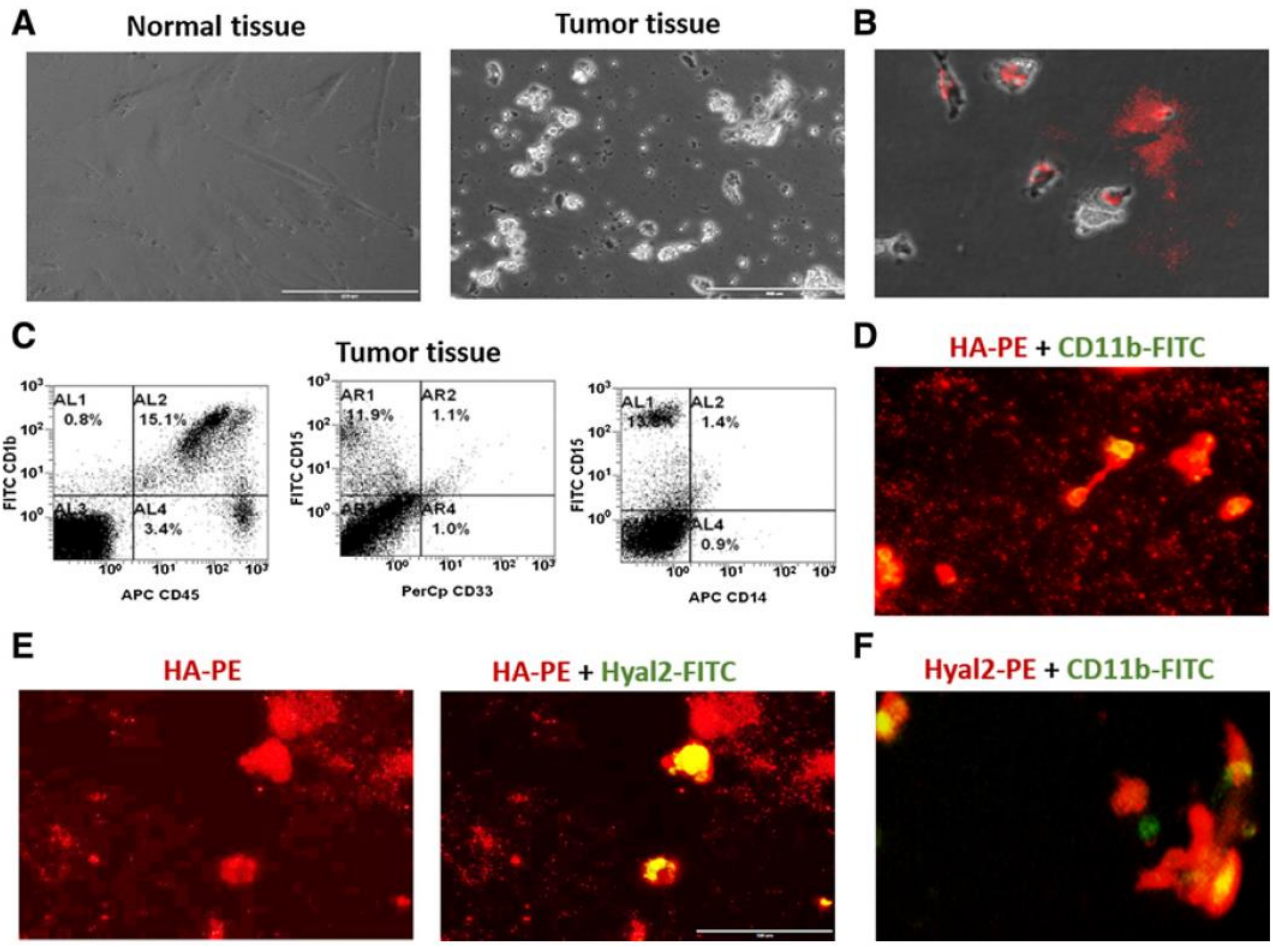
Key Data

Degradation of HA in human tumor tissue is associated with the presence of tumor-infiltrating CD11b⁺Hyal2⁺ myeloid cells



A, Representative bright-field images of normal bladder tissue (left) and bladder cancer tissue (right) from the same patient are shown. B, Colocalization of tumor-infiltrating cells and fragmented tumor produced HA (red). A representative image is shown. C, Flow cytometric analysis of bladder cancer tissue. Tumor tissue from cancer patients was digested with a collagenase cocktail to prepare single-cell tumor suspension. The single-cell suspension was co-stained with CD11b-FITC, CD45-APC, CD33-PerCp, CD15-FITC, CD14-APC antibodies, and analyzed by flow cytometry. Representative images are shown. D, Colocalization of tumor-infiltrating CD11b myeloid cells and fragmented tumor-produced HA. Representative image of CD11b (green) and HA (red) in bladder cancer tissue is shown. E, Visualization of tumor-produced HA and tumor associated Hyal2-expressing cells. The human cancer tissue slices were cultured for 5 days. Nonadherent cells were carefully removed, washed with PBS, and fixed with 4% formaldehyde. To visualize the tumor-produced HA, biotinylated HA-binding protein and PE-labeled streptavidin were subsequently added. Representative images of HA (red) and Hyal2 (green) are shown. F, Detection of tumor-infiltrating CD11b⁺Hyal2⁺ myeloid cells. Representative image of CD11b (green) and Hyal2 (red) in bladder cancer tissue is shown.

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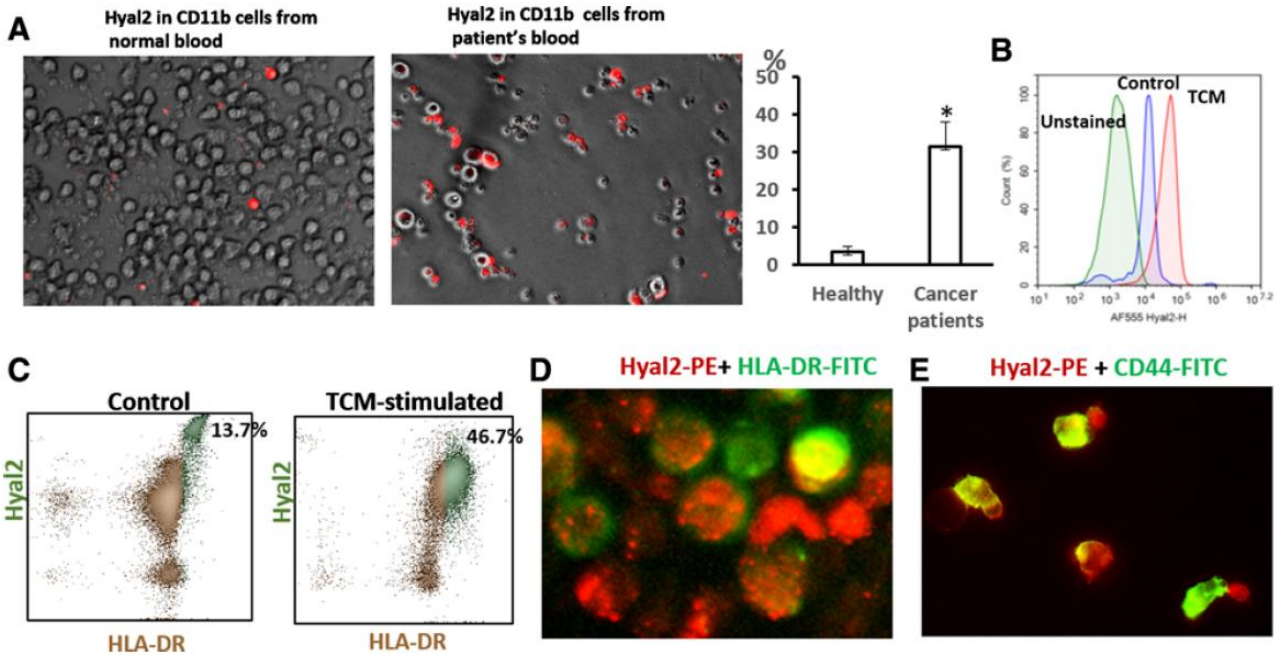
Identification of Hyal2-expressing myeloid cell subsets in peripheral blood from patients with bladder cancer



A, Upregulated expression of Hyal2 by peripheral blood-derived CD11b myeloid cells from patients with cancer. CD11b myeloid cells were isolated from the peripheral blood of normal individuals or cancer patients using magnetic beads, stained with anti-Hyal2-PE antibodies, and analyzed by immunofluorescent microscopy. The percent of Hyal2þ cells was evaluated using an immunofluorescent imaging microscope. Average means SD are shown. , $P < 0.05$. B and C, Analysis of Hyal2 expression in blood-derived myeloid cells using flow cytometry. CD11b myeloid cells were isolated from the peripheral blood of patients with cancer and cultured in complete culture medium for 48 hours in the presence or absence of TCM. T24 tumor cell–derived culture supernatant was a source of TCM in these experiments. Collected cells were washed with PBS and stained with anti-Hyal2 Abs (B), anti-Hyal2, and anti-HLA-DR abs (C). The expression of indicated markers was measured using flowcytometry. Representative images are shown. D, Analysis of Hyal2 localization in TCM-stimulated myeloid cells using immunofluorescent microscopy. TCM-stimulated myeloid cells were prepared as indicated above and stained with anti-Hyal2-PE (red) and HLA-FITC (green) antibodies. Representative images are shown. E, Hyal2þ myeloid cells co-express CD44. CD11b myeloid cells were isolated from the peripheral blood of patients with cancer using magnetic beads. TCM-stimulated myeloid cells were prepared as indicated above and stained with CD44-FITC and anti-Hyal2-PE. A representative image is shown.

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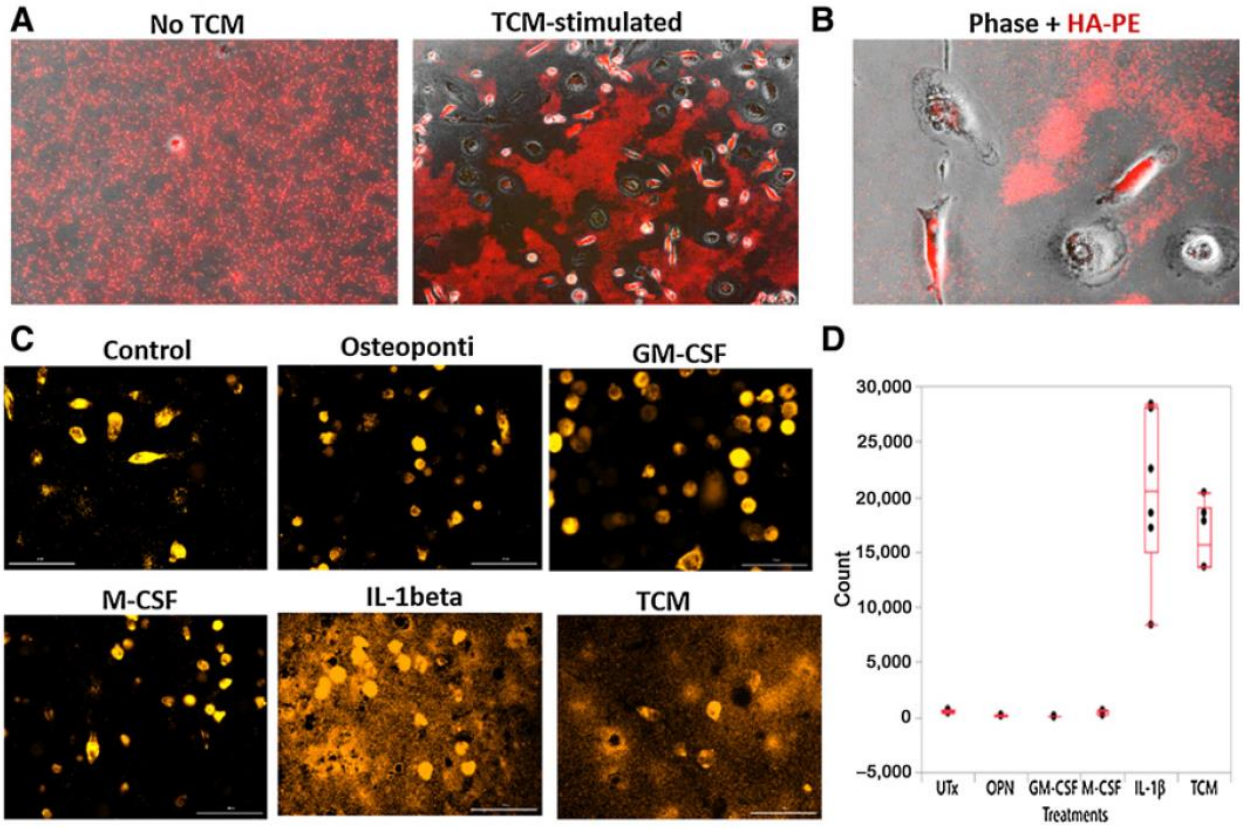
IL1b stimulates HA-degrading activity of Hyal2+ myeloid cells.



A, Stimulation of peripheral blood-derived myeloid cells with TCM promotes the degradation of extracellular HA. Twenty-four–well cell culture plates were precoated with sterile commercial HA (MW 200 kDa). CD11b myeloid cells isolated from peripheral blood of patients with cancer were cultured in the absence (left) or the presence of TCM (right). On day 10, cell cultures were fixed with 4% formaldehyde and stained for the HA (red). Representative images of control and TCM-treated myeloid cells cultured with HA are shown. B, Detection of intracellular HA in TCM-stimulated myeloid cells. CD11b myeloid cells isolated from peripheral blood of patients with bladder cancer were added to the wells with precoated commercial HA. Cells were cultured in the presence of TCM for 10 days. A representative image is shown. C, IL1b stimulates the HA-degrading activity of Hyal2+ myeloid cells. Hyal2+ were directly from peripheral blood of the cancer patient and were cultured in a 24-well plate that was precoated with commercial HA (MW 200 kDa) in complete culture medium in presence of bladder TCM, or GM-CSF (50 ng/mL), M-CSF (50 ng/mL), osteopontin (OPN; 50 ng/mL), IL1b (50 ng/mL) or none (Utx). Supernatant from the patient's bladder tumor tissue culture was a source of TCM in these experiments. HA was visualized on day 10 as described in Materials and Methods using immunofluorescent microscopy. D, Quantification of HA fragments in cytokine-treated Hyal2+ cells. Image analysis was done using Gen 5 Prime v 3.08 software (Biotek Instruments).