Tissue specific splicing program of hMENA in the dialogue between tumor cells and stroma: a powerful indicator of mesenchymal traits in NSCLC and PDAC

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ABSTRACT

Deciphering the complexity of the tumor microenvironment (TME) is essential to unveil mechanisms of therapy resistance and to develop novel microenvironment-related anti-tumor treatment. Actin cytoskeleton dynamics act as platforms for gene regulation and key signaling transduction pathways involved in the cross-talk between tumor cells and TME. We have demonstrated that the actin regulator hMENA controls the Serum Response Factor (SRF) activity, and the expression of its target gene β1 Integrin, by affecting the G-Actin/F-Actin ratio, critical for the nuclear localization of the SRF co-factor myocardin related transcription factor 1 (MRTF1). The splicing of hMENA generates two alternatively expressed isoforms, with hMENA^{11a} and hMENAΔv6 respectively inhibiting or increasing cell invasiveness, SMAD2-mediated-TGFβ signaling, Endothelin1/β-arrestin1–induced invadopodial activity, activation of β1 integrin signaling and the secretion of several key extracellular matrix (ECM) proteins. hMENA isoform expression pattern is a powerful prognostic factor in early non-small-cell lung cancer (NSCLC) and pancreatic cancer patients. By evaluating the expression of fibronectin (FN) in the stroma, we found that early node-negative NSCLC patients show a prolonged disease-free survival (DFS) when expressing high hMENA^{11a}/low stromal FN, indicating the need to pay serious attention to the patterns of protein expression in the stroma.

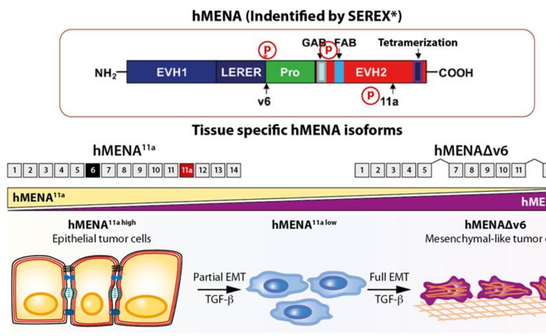
Herein, we investigate the role of hMENA isoforms in the cross-talk of tumor cells and cancer-associated fibroblasts (CAF). hMENA/hMENAΔv6 are overexpressed in CAFs with respect to normal fibroblasts and promote CAF pro-tumoral functional activity in PDAC and NSCLC. We have identified a novel function of hMENA in regulating tumour/stroma cross-talk via the modulation of Gas6-Axl signaling, crucial in EMT, drug resistance and immune evasion. CAFs over-expressing hMENAΔv6 secrete the Axl ligand Gas6, favoring the invasiveness of Axl-expressing NSCLC and PDAC cells. Notably, in tumor cells hMENA/hMENAΔv6 regulate Axl expression sustaining the paracrine Gas6-Axl signaling. From a clinical point of view, a high hMENA/Gas6/Axl gene expression signature is associated with a poor prognosis in NSCLC and PDAC patients.

Our findings indicate that the alternative splicing of hMENA is crucial in the reciprocal signaling between tumor cells and CAF, regulates the ECM composition and cytokine milieu and we suggest may affect the spatial distribution of T cells and tertiary lymphoid structures. The expression pattern of hMENA isoforms in both tumor cells and CAFs may identify tumor mesenchymal traits that emerged as a common feature of T cell exclusion and of different signatures of resistance to immune checkpoint blockade (ICB).

We are characterizing the role of hMENA in immunomodulatory properties of CAF subtypes, by setting up innovative 3D models based on the bio-printing of CAFs, T lymphocytes and tumor cells in predefined ECM. By using the Nanostring platform we are evaluating the role of the hMENA expression pattern as a surrogate marker of a TME of responder or non-responder ICB treated patients

BACKGROUND

The hMENA isoform-specific roles in cancer



Expression	Function
• Epithelial cells • Absent in invasive cells • Down-regulated by TGFβ1 during EMT	• Promotes cell-cell adhesion • Sustains E-Cadherin expressiom • Anti-apoptotic expression • Pro-proliferative • Anti-invasive • Reduces ECM components (Fibronectin and MMPs) and MMP activity • Inhibits TGFβ1 signaling • Inhibits β1 integrin signaling

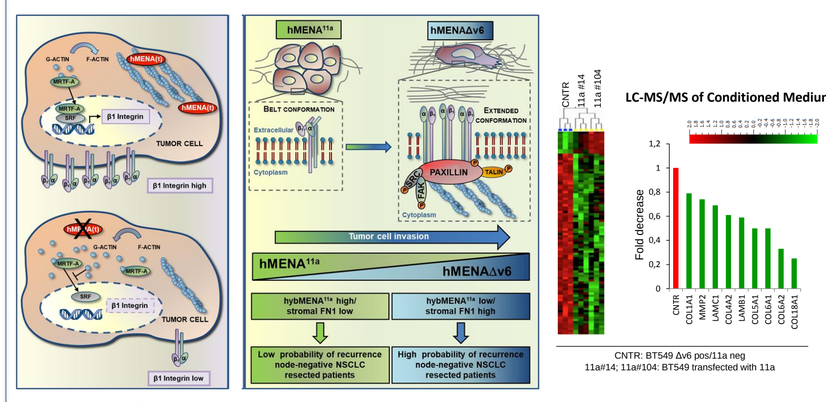
Relationship to outcome
hMENA-hMENA^{11a} ⟶ Favourable outcomes

Expression	Function
• Mesenchymal "like" epithelial cells • Mesenchymal cells • Over-expressed by TGFβ1 during EMT • Up-regulated by TGFβ1, β-catenin, ET1	• Sustains Vimentin expression • Increases cell invasiveness • invadopodia formation and function • Promotes TGFβ1 induced EMT • Increases β1 integrin signaling • Increases ECM components

Relationship to outcome
hMENA-hMENAΔv6 ⟶ Poor outcomes

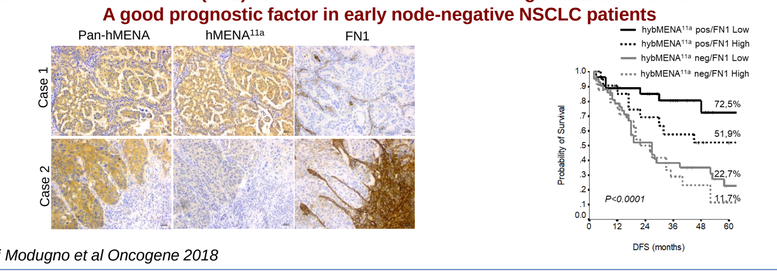
Di Modugno et al. Int J Cancer. 2004; Di Modugno et al. Cancer Res 2007; Di Modugno et al. PNAS 2012; Bria, Di Modugno et al. Oncotarget 2014; Trono et al. Oncogene 2016; Melchionna et al OncoImmunology 2016; Sistigu, Di Modugno, Cytokine & Growth Factor Reviews, 2017: Di Modugno, Spada et al., Oncogene 2018

hMENA isoform impact NSCLC patient outcome through fibronectin/β1 integrin axis



CNTR: BT549 Δv6 pos/11a neg
11a#14; 11a#104: BT549 transfected with 11a

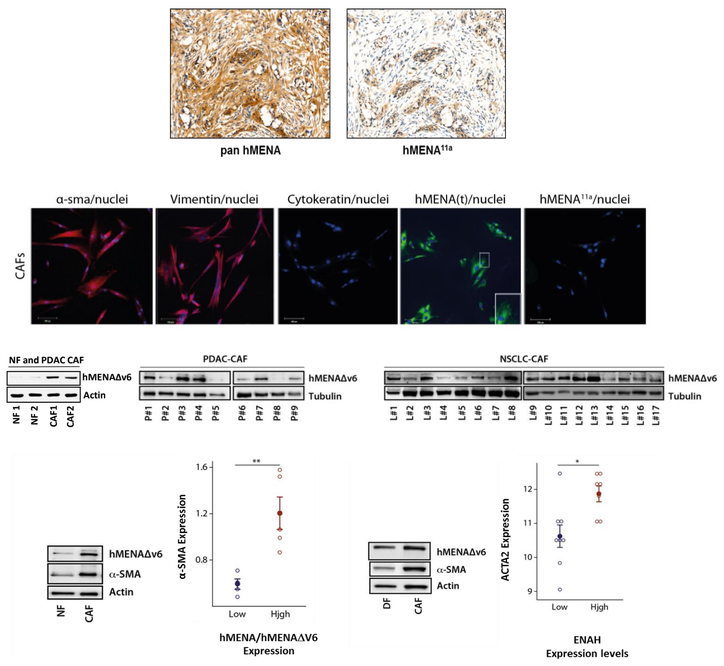
Low level of fibronectin (FN1) in the stroma correlates with high hMENA^{11a} in tumor cells: A good prognostic factor in early node-negative NSCLC patients



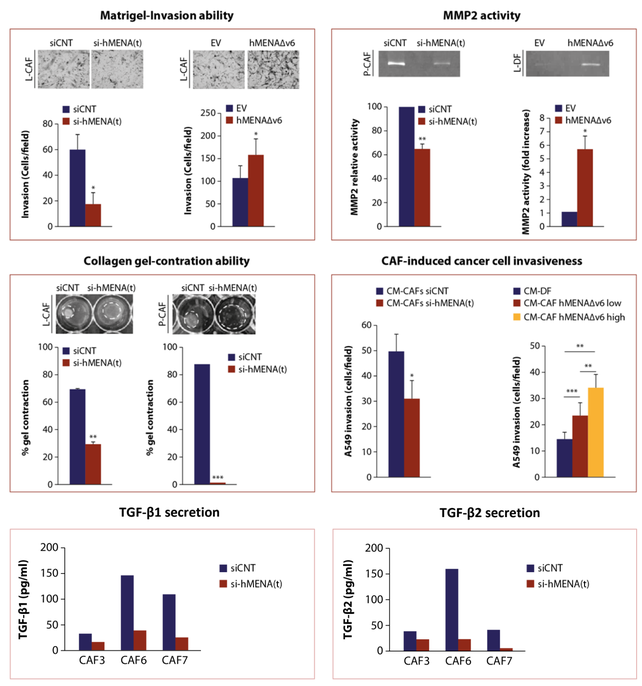
Di Modugno et al Oncogene 2018

RESULTS

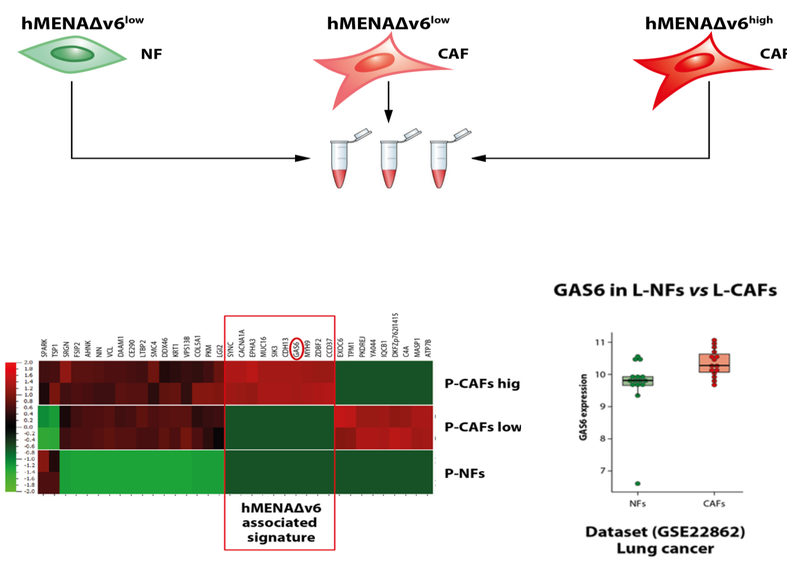
CAFs have high level of hMENAΔv6 isoform



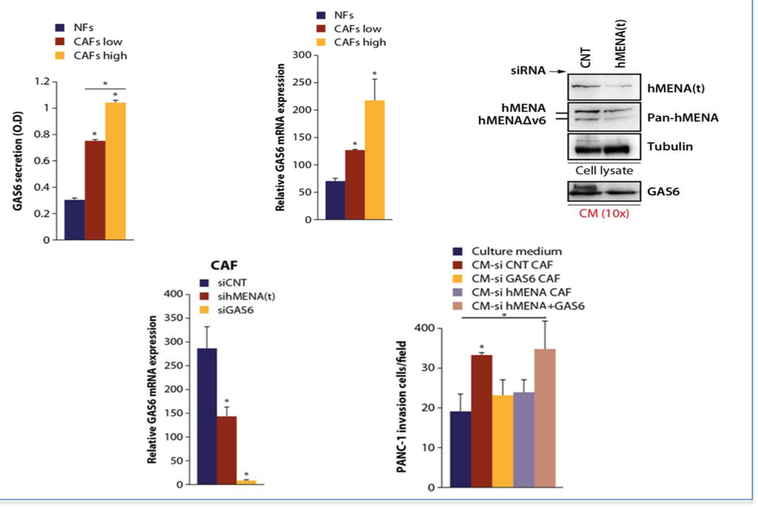
hMENAΔv6 isoform is crucial for CAF activation



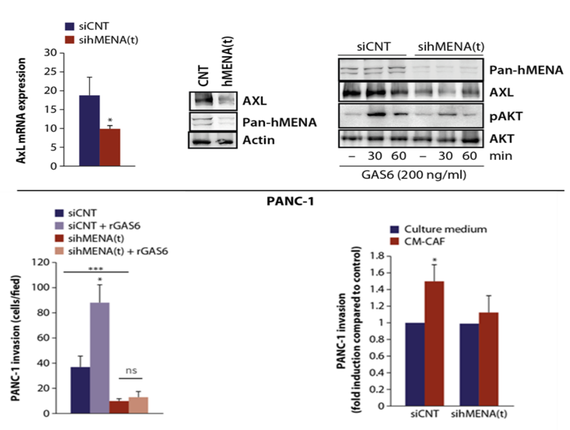
Identification of GAS6 from hMENAΔv6-associated signature in CAFs



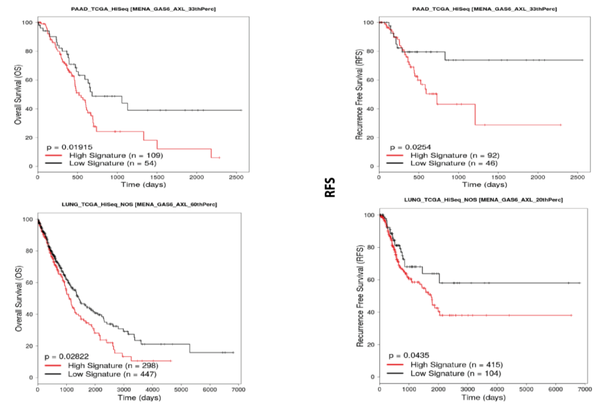
hMENA/hMENAΔv6 expression in CAFs favours tumor cell invasion *via* GAS6 modulation



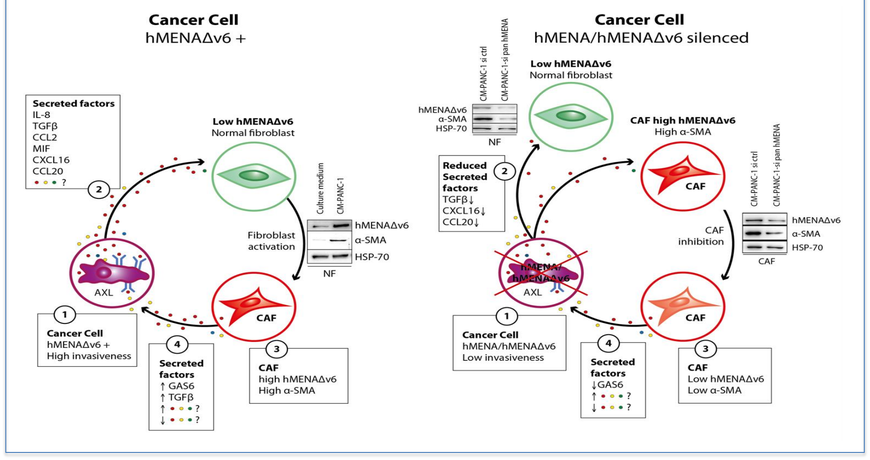
hMENA/hMENAΔv6 expression in cancer cells sustains AXL/GAS6 axis



Combined expression of hMENA, Axl and GAS6 as a prognostic gene signature that predicts patient outcome



Tumor secretoma increases hMENAΔv6 expression and activation of NF. hMENA is crucial in the crosstalk between cancer cell and CAF



CONCLUSIONS

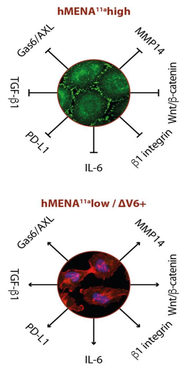
We have identified a novel role for hMENA in mediating the interaction between tumor cells and CAFs not only by promoting CAF activation but also by regulating the Gas6-Axl axis, key signaling pathway in EMT, drug resistance and immune evasion

We indicate that the network based on hMENA/GAS6/Axl expression may represent novel prognostic and therapeutic target

We suggest that the pattern of hMENA isoform expression in both tumor cells and CAFs may reveal tumor enriched mesenchymal traits which may identify tumor subtype for tailored therapies

FUTURE PERSPECTIVE

hMENA/hMENAΔv6 drive tumor and stroma enrichment of mesenchymal traits, hallmark of T cell exclusion and immune evasion

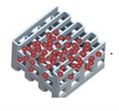


hMENA splicing program as a platform to identify theranostic biomarkers, focus on ICB in NSCLC by:

-Nanostring platform on tumor tissues of ICB responder or non responder patients

-Immune related cytokine profiling by Bioplex of CAF-tumor cell co-coltures

-Innovative 3D models (Bioprint) for the co-colture of tumor cells, CAF and T lymphocytes embedded in ECM extracted from human lung cancer tissues



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