hMENA^{11a} restrains PD-L1 expression by affecting IFN signaling pathway in NSCLC

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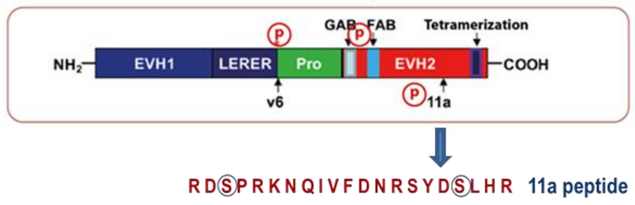
BACKGROUND

hMENA, an actin regulatory protein of the ENA/VASP family, plays key roles in cellular processes that rely on actin cytoskeleton dynamics (1). The hMENA gene undergoes splicing process that gives rise to different isoforms, and our group has identified hMENA^{11a} (2) and hMENADv6, two alternatively expressed variants with opposite functions in tumor proliferation and invasion (3). The pro-proliferative hMENA^{11a} isoform derives from the inclusion of the extra exon 11a, that generates a 21 amino acid peptide carrying three putative phosphorylation sites, in a site adjacent to the F-actin and G-actin binding sites (2). In breast cancer hMENA is downstream to EGFR family signaling, correlates with HER2 and the activation status of AKT and its concomitant overexpression with HER2 identifies a subgroup of patients with a worst prognosis (4). Recently, hMENA^{11a} has emerged as a key signaling hub that participates in the balance of pro- and anti-apoptotic proteins, and contributes to mechanisms of resistance to PI3K inhibitors sustaining survival signaling pathways related to HER3. PI3K inhibition induces hMENA^{11a} phosphorylation, mediated by a still unknown kinase (5). In pancreatic cancer we have demonstrated that TGF-β1 down-regulates hMENA^{11a}, whereas increases hMENA and hMENADv6 expression (6). Of clinical relevance the absence of hMENA^{11a} correlates with poor outcome in a subset of pan-hMENA-positive tumors in early lung cancer and in PDAC patients (6,7).

hMENA (identified by SEREX)



hMENA^{11a} :

- Sustains E-cadherin expression and cell junction integrity
- Is down-regulated by TGFβ, during EMT
- Is phosphorylated by EGF and NRG
- Affects cell proliferation in breast cancer cell lines
- Correlates with HER2 overexpression, Ki67 and an activation status of AKT and MAPK in breast tumors
- Cooperates with ErbB receptor family and, when concomitantly expressed with HER2, identifies a subset of breast cancer patients with a worse prognosis
- Sustains HER3 pathway activation and resistance to PI3K inhibition
- Is a predictor of disease-free and cancer-specific survival in resected, node-negative, NSCLC and in PDAC patients

ABSTRACT

Programmed Death-1(PD-1) and PD-Ligand-1 (PD-L1) blockade have yielded promising clinical effects in NSCLC, and PD-L1 expression in either tumor cells or tumor-infiltrating immune cells identifies patients who are more likely to respond to immune-checkpoint blockade (ICB). Thus, understanding the regulation mechanisms of PD-L1 expression is of great relevance.

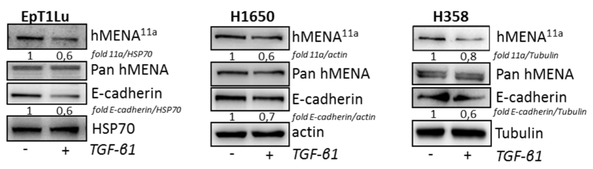
hMENA is a member of the Ena/VASP family, actin cytoskeleton regulatory proteins. hMENA undergoes alternative splicing and our group has identified two splicing-derived hMENA isoforms inversely associated to EMT process: hMENA^{11a} and hMENADv6 associated with epithelial or mesenchymal-like cells respectively (3). hMENA^{11a} isoform correlates with E-cadherin expression and contributes to the maintenance of cell-cell junction integrity whereas its downregulation perturbs cell junction integrity and is associated with the up-regulation of molecules involved in apoptosis (5,6). Recently, it has been reported that PD-L1 expression correlates with a low expression of E-cadherin and a mesenchymal-like phenotype in human lung adenocarcinomas (8).

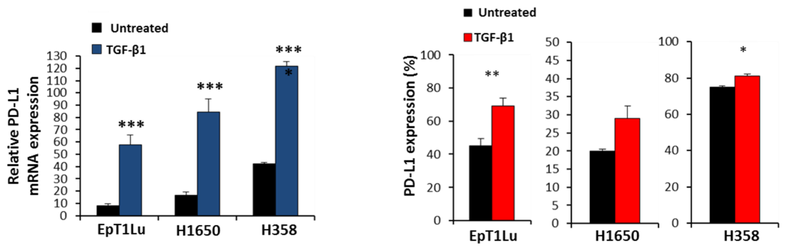
Herein, we show that the critical EMT regulator TGFβ decreases hMENA^{11a} levels along with E-cadherin in NSCLC cells, and increases PD-L1 at mRNA and protein levels, suggesting that low levels of hMENA^{11a} expression associate with early EMT-related events. To investigate the effects of hMENA^{11a} silencing, we performed RNA-Seq in epithelial H1650 cell line silenced for hMENA^{11a} by a pool of specific siRNAs. The transcriptional profile of these cells confirms the decrease of E-cadherin and show that the silencing of this isoform *per se* increases PD-L1 transcripts. We found the up-regulation of a list of immune-related genes, such as MHC class I and II, including co-stimulatory molecules, and cytokines such as IL6 and IL8. Gene Ontology enrichment analysis revealed that a major fraction of transcripts up-regulated in cells 11a silenced code proteins significantly associated with interferon-mediated signaling pathways. IFN signaling, traditionally considered a key factor in anticancer immunity, has recently also involved in the immune escape, by enabling the up-regulation of PD-L1 in tumor, immune and stromal cells and has been recently associated with ICB resistance. We hypothesize that hMENA^{11a} loss causes a critical perturbation of cell-cell junction integrity that leads to IFN signaling activation and herein we investigated the role of hMENA^{11a} in the regulation of PD-L1 IFN-mediated. We found a sustained STAT1 phosphorylation status upon hMENA^{11a} silencing in NSCLC cells and we analyzed the JAK/STAT/IRF1 axis as it is reported to regulate PD-L1 expression upon IFN signaling. By immunofluorescence analysis, we found that the PD-L1 transcriptional activator IRF1 is predominantly located in the nucleus of cells depleted for hMENA^{11a}, while nuclear levels of the PD-L1 transcriptional repressor IRF2 strongly decrease, suggesting that hMENA^{11a} plays a critical role in the regulation of PD-L1 transcription by regulating IRF1/IRF2 nuclear-cytoplasmic shuttle. Altogether, these data indicate that hMENA^{11a} restrains PD-L1 expression by affecting IFN signaling in NSCLC, and suggest that the pattern of this isoform expression may be crucial in maintaining off IFN signaling status. These data highlight the need to investigate the potential role of hMENA^{11a} as a biomarker useful for the identification of subsets of patients likely to benefit from ICB in NSCLC. We are analyzing the correlation between hMENA^{11a} and PD-L1 expression and IFN signaling activation by Nanostring platform in RNA from biopsy specimens from patients responding and not responding to ICB.

1) Bear et al, Cell 2000; 2) Di Modugno et al, Cancer Res 2007; 3) Di Modugno et al, PNAS 2012; 4) Di Modugno et al, Plos One 2010; 5) Trono et al, Oncogene 2016 6) Melchionna et al, OncoImmunology 2016; 7) Bria, Di Modugno et al, Oncotarget 2014; 8) Lou et al, Clin Cancer Res 2016

RESULTS

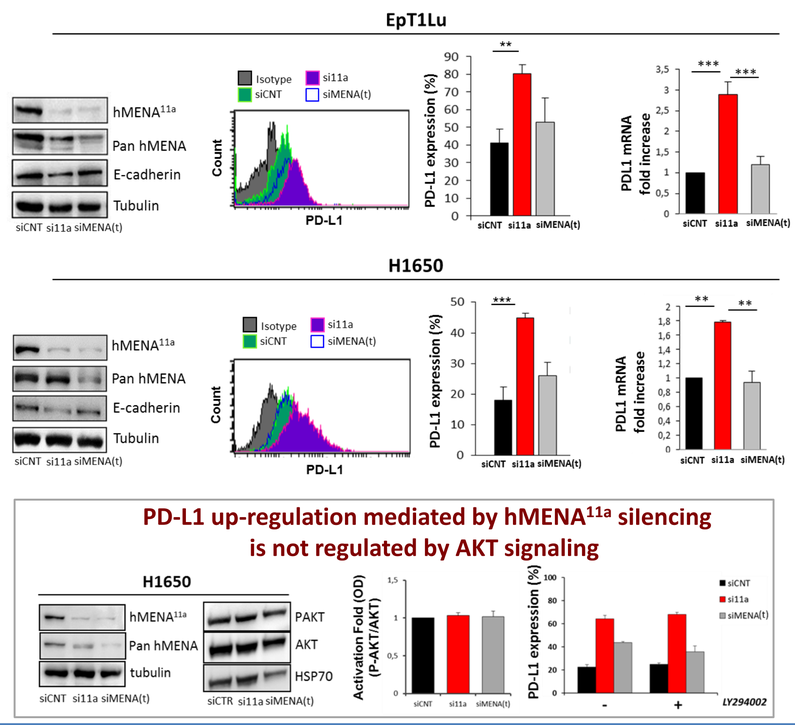
TGF-β1 treatment decreases hMENA^{11a} along with E-cadherin expression and increases PD-L1 at mRNA and protein levels in epithelial NSCLC cell lines



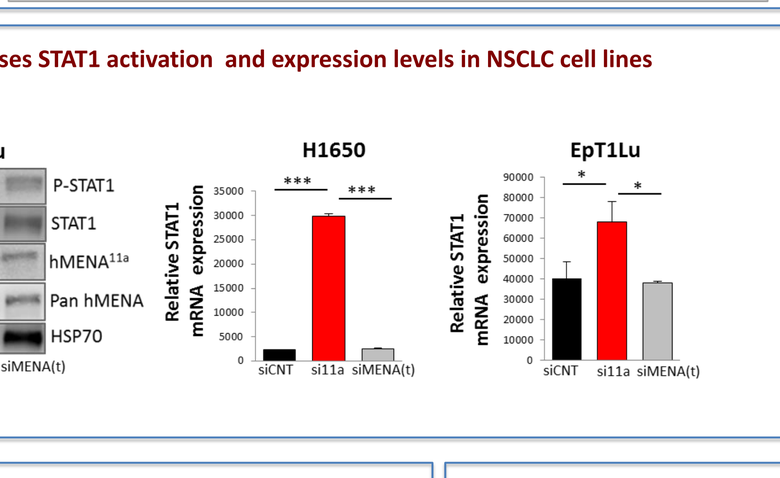


EpT1Lu: patient-derived NSCLC epithelial cell line

hMENA^{11a} silencing decreases E-cadherin and increases PD-L1 at protein and mRNA levels in NSCLC cell lines

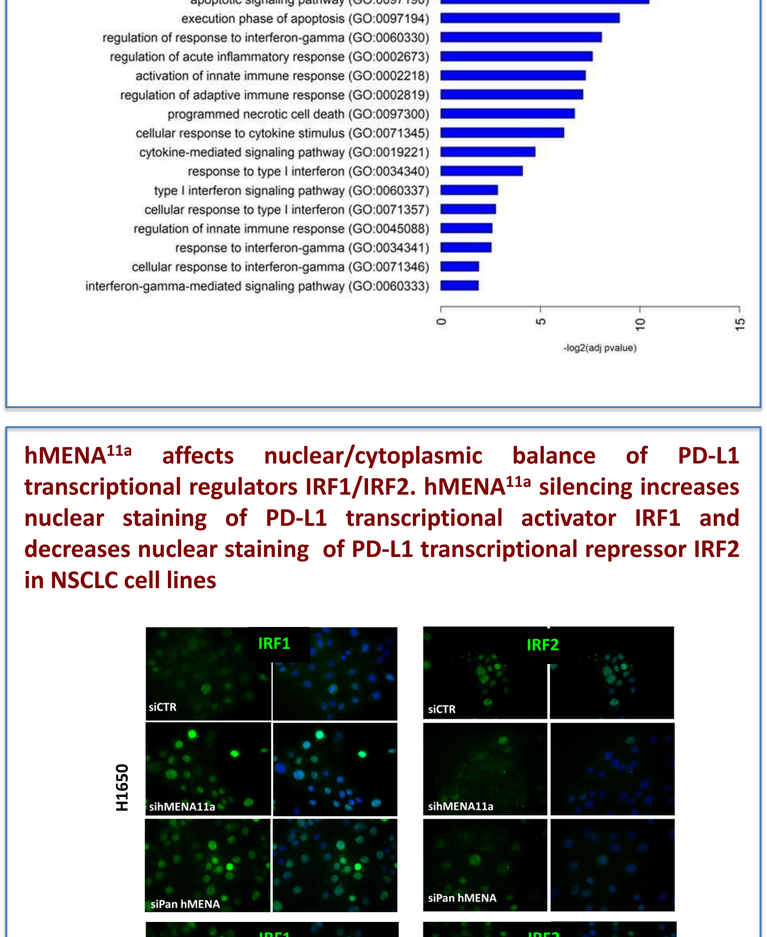


PD-L1 up-regulation mediated by hMENA^{11a} silencing is not regulated by AKT signaling



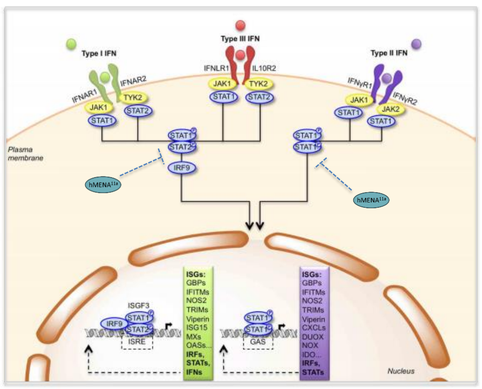
hMENA^{11a} silencing up-regulates transcripts of genes related to IFN-signaling pathways in H1650 cell line

Gene Ontology (GO) term enrichment analysis of RNA-seq data: up-regulated GO Biological Processes in H1650 si11a cells



WORKING HYPOTHESIS

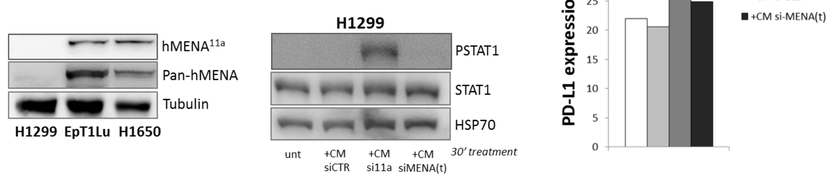
hMENA^{11a} restrains IFN signaling pathway activation



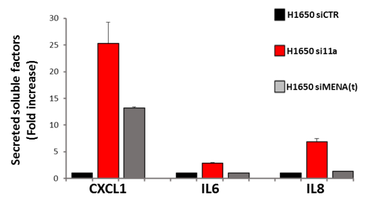
Adapted by ***Dussurget et al., Front. Cell. Infect. Microbiol. 2014***

hMENA^{11a} silencing increases STAT1 activation and expression levels in NSCLC cell lines

Conditioned medium (CM) of H1650 ^{11a} silenced cells induces STAT1 activation and PD-L1 up-regulation in H1299 hMENA^{11a} negative cells



hMENA^{11a} silencing increases production of CXCL1, IL6 and IL8



hMENA^{11a} affects nuclear/cytoplasmic balance of PD-L1 transcriptional regulators IRF1/IRF2. hMENA^{11a} silencing increases nuclear staining of PD-L1 transcriptional activator IRF1 and decreases nuclear staining of PD-L1 transcriptional repressor IRF2 in NSCLC cell lines

CONCLUSIONS

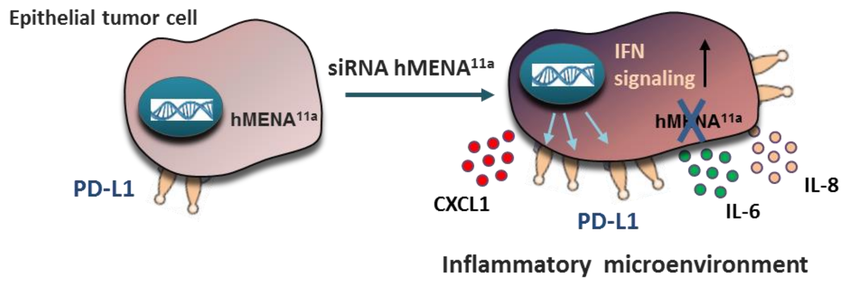
Our results indicate that:

- TGFβ decreases hMENA^{11a} levels along with E-cadherin in NSCLC cells, and increases PD-L1 at mRNA and protein levels.
- hMENA^{11a} silencing decreases E-cadherin and increases PD-L1 at protein and mRNA levels in NSCLC cell lines, independently from AKT signaling.
- hMENA^{11a} silencing in an epithelial NSCLC cell line up-regulates transcripts coding proteins significantly associated with interferon-mediated signaling pathways.
- hMENA^{11a} silencing increases STAT1 activation and expression levels in NSCLC.
- Soluble factors produced by hMENA^{11a} silenced cells induce STAT1 activation and PD-L1 upregulation in one hMENA11a negative NSCLC cell line.
- hMENA^{11a} affects nucleus/cytoplasm shuttle of transcriptional factors IRF1 and IRF2, activator and repressor of PD-L1 transcription, respectively.

We hypothesize that hMENA^{11a} loss causes a critical perturbation of cell-cell junction integrity that leads to IFN signaling activation.
We suggest that hMENA^{11a} restrains PD-L1 expression by affecting IFN signaling in NSCLC and may contribute to resistance/sensitivity to inhibitory checkpoint blockade (ICB).

We propose hMENA^{11a} along with the other isoforms as potential biomarker useful for the identification of subsets of patients likely to benefit from ICB in NSCLC

hMENA11a as regulator of PD-L1 expression



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