Human enabled homolog (ENAH, also known as hMENA), an actin regulatory protein of the enabled/vasodilator-stimulated phosphoprotein (ENA/VASP) family, has a crucial role in cellular processes that rely on actin cytoskeleton dynamics. hMENA is absent in normal breast but is overexpressed in breast tumors, where its expression correlates with the human epidermal growth factor receptor (HER) 2-positive, estrogen receptor/progesterone receptor (ER/PgR)-negative, and high-Ki67 phenotype. hMENA crosstalks with HER family signaling and its concomitant overexpression with HER2 identifies a subgroup of patients with the worst prognosis.1

The hMENA gene undergoes a splicing process that gives rise to multiple isoforms that are expressed in specific tissues and cell types.2 Among these isoforms, our group has identified 2 alternatively expressed variants, one with the inclusion of exon 11a (hMENA11a) and a proliferative,3 anti-invasive,4 role in cancer, and the other with exclusion of exon 6 (hMENA/hMENA 11a), is an invasive role in breast and lung cancer.5,6 Inclusion of the additional exon 11a is regulated by different splicing factors8 and occurs in a site adjacent to the F-actin and G-actin binding sites in the enabled/vasodilator-stimulated phosphoprotein homology 2 (Evh2) domain. Three putative phosphorylation sites (serine 3, serine 18, and tyrosine 16) are located in the 21 amino acids encoded by the 11a exon although the corresponding kinases are still unknown.9 hMENA11a, together with hMENA (hMENA/hMENA11a), is expressed in epithelial breast tumor cells. Epidermal growth factor (EGF) and neuregulin (NRG-1), as well as HER2 upregulation and activity, increase the expression levels of hMENA and hMENA11a, and phosphorylation of only hMENA11a.3 Conversely, trastuzumab decreases hMENA expression and reduces hMENA11a phosphorylation.1 Depletion of hMENA/hMENA11a decreases HER3 phosphorylation, inhibits EGF- and NRG-mediated activation of epidermal growth factor receptor (EGFR, also known as HER1) and HER2, and counteracts growth factor-mediated cell proliferation.1

Actin binding proteins regulate different apoptosis pathways, and remodeling of the actin cytoskeleton favors evasion by tumor cells of normal apoptotic signaling. Currently no data are available on the role of hMENA11a in signaling related to cell survival and apoptosis.

Recently, we highlighted a novel role for hMENA11a.7 We designed a reverse phase protein array (RPPA) assay to investigate whether hMENA11a plays a role in oncogenic signaling linked to cell proliferation and survival (Fig. 1A). Notably, the heatmap generated by the RPPA analysis highlights how specific silencing of hMENA11a dramatically switches off molecules relevant to cancer cell survival, such as survivin and, among the tyrosine kinases, EGFR, HER2, and HER3. Conversely, silencing of hMENA11a switches on molecules linked to apoptosis such as cleaved forms of poly(ADP-ribose) polymerase (PARP) and caspase 9 (CASP9) (Fig. 1A). hMENA11a silencing impaired the NRG-1-mediated activation of HER3 in HER2 overexpressing breast cancer cell lines, suggesting that NRG-1 was no longer able to activate HER3 in cells lacking hMENA11a.

Thus, we reasoned that a correlation between hMENA11a and activation of HER3 may also occur in vivo. Indeed, we found a significant (p<0.0001) correlation between P-HER3 and hMENA11a in a cohort of primary HER2-positive breast tumors and showed that 95% of cases with a 2+ or 3+ P-HER3 score were also positive for hMENA11a. Interestingly, strong and membranous staining of hMENA11a was found in cells that were highly positive for P-HER3 whereas cells

**hMENA**

**hMENA isoform sending survival signals**

Paola Trono, Francesca Di Modugno, and Paola Nisticò

Department of Research, Advanced Diagnostics and Technological Innovation, Regina Elena National Cancer Institute, Rome, Italy

**ABSTRACT**

Human MENA11a (hMENA11a), an epithelial-associated isoform of the actin binding protein enabled homolog (ENAH, also known as mammalian ENA [MENA]), is upregulated and phosphorylated following the activation of human epidermal growth factor receptor (HER) 1, HER2, and HER3. Here, we reveal a novel role of this isoform in sustaining cell survival and propose hMENA11a as a marker of HER3 activation and resistance to phosphatidylinositol-3-kinase inhibition therapies.

**Abbreviations:** BIM, Bcl2-interacting mediator of cell death; CASP9, caspase 9; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ENAH, enabled homolog; ENA/VASP, enabled/vasodilator-stimulated phosphoprotein; ER, estrogen receptor; FOXO3a, Forkhead box O3a; HER, human epidermal growth factor receptor; MENA, mammalian enabled; PgR, progesterone receptor; NRG, neuregulin; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol-3-kinase; RPPA, reverse phase protein array
that scored low for P-HER3 showed a cytoplasmic localization of hMENA11a, suggesting that when HER3 is activated hMENA11a has a membranous localization. A correlation between HER3 and hMENA11a expression was also found (p \leq 0.008).

Recently, the overexpression and activation of HER3 has been reported to be crucial for mechanisms of cell resistance to different therapies, including phosphatidylinositol-3-kinase (PI3K) inhibitors. Thus, our study logically turned to investigate whether hMENA11a has a role in HER3-based resistance mechanisms to PI3K inhibition in HER2 overexpressing breast cancer cell lines. Indeed, we found that HER3 upregulation and activation induced by the PI3K inhibitor BEZ235 were impaired in cells silenced for hMENA11a and treated with PI3K inhibitors; FOXO3a is sequestered in the cytoplasm and does not shuttle into the nucleus and HER3 is not upregulated, rendering cells sensitive to therapy.

Figure 1. hMENA11a delivers survival signals and promotes resistance to PI3K inhibition. (A) Overexpression of hMENA11a participates in receptor tyrosine kinase (RTK) and survival pathway activation. Specific silencing of hMENA11a inhibits RTK activation (i.e., P-HER3), favoring cell apoptosis. (B) Treatment of HER2+ breast cancer cell lines with PI3K inhibitors determines FOXO3a nuclear translocation and HER3 upregulation, a mechanism involved in therapy resistance. PI3K inhibitors induce phosphorylation of hMENA11a. In cells that are specifically silenced for hMENA11a and treated with PI3K inhibitors, FOXO3a is sequestered in the cytoplasm and does not shuttle into the nucleus and HER3 is not upregulated, rendering cells sensitive to therapy. BIM, Bcl2-interacting mediator of cell death; CASP9, caspase 9; FOXO3a, Forkhead box O3a; hMENA, human MENA; P-HER3, phospho-human epidermal growth factor receptor 3; RTK, receptor tyrosine kinase; P-AKT, phospho-protein kinase B; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol-3-kinase.

although further efforts are needed to unambiguously place hMENA11a in druggable signaling networks, hMENA11a is emerging as a key signaling hub that is able to intersect the axis connecting EGFR family proteins to downstream molecules such as PI3K. The critical contribution of hMENA11a to cell resistance to PI3K inhibition highlights the need to shut down hMENA11a signaling.
when designing more efficacious breast cancer targeted therapies.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**References**

1. Di Modugno F, Mottolese M, DeMonte L, Trono P, Balsamo M, Conidi A, Melucci E, Terrenato I, Belleudi F, Torrisi MR, et al. The cooperation between hMena overexpression and HER2 signaling in breast cancer. PLoS One 2010; 5:e15852; PMID:21209853; http://dx.doi.org/10.1371/journal.pone.0015852
2. Gertler F, Condeelis J. Metastasis: tumor cells becoming MENAcing. Trends Cell Biol 2011; 2:81–90; http://dx.doi.org/10.1016/j.tcb.2010.10.001
3. Di Modugno F, DeMonte L, Balsamo M, Bronzi G, Nicotra MR, Alessio M, Jager E, Condeelis JS, Santoni A, Natali PG, et al. Molecular cloning of hMena (ENAH) and its splice variant hMena 11a: epidermal growth factor increases their expression and stimulates hMena 11a phosphorylation in breast cancer cell lines. Cancer Res 2007; 67:2657–2665; PMID:17363586; http://dx.doi.org/10.1158/0008-5472.CAN-06-1997
4. Di Modugno F, Iapicca P, Boudreau A, Mottolese M, Terrenato I, Perracchio L, Carstens RP, Santoni A, Bissell MJ, et al. Splicing program of human MENA produces a previously undescribed isoform associated with invasive, mesenchymal-like breast tumors. Proc Natl Acad Sci USA 2012; 109:19280–19285; PMID:23129656; http://dx.doi.org/10.1073/pnas.1214394109
5. Bria E, Di Modugno F, Sperduti I, Iapicca P, Visca P, Alessandri G, Antoniani B, Pilotto S, Ludovini V, Vannucci J, et al. Prognostic impact of alternative splicing-derived hMENA isoforms in resected, node-negative, non-small-cell lung cancer. Oncotarget 2014; 5(22):11054–63; PMID:25373410; http://dx.doi.org/10.18632/oncotarget.2609
6. Mallinjoud P, Villemin JP, Mortada H, Polay Espinoza M, Desmet FO, Samaan S, Chautard E, Tranchezent LC, Auboueuf D. Endothelial, epithelial, and fibroblast cells exhibit specific splicing programs independently of their tissue of origin. Genome Res 2014; 3:511–21; http://dx.doi.org/10.1101/gr.162933.113
7. Trono P, Di Modugno F, Circo R, Spada S, Di Benedetto A, Melchionna R, Palermo B, Matteoni S, Soddu S, Mottolese M, et al. hMENA11a contributes to HER3-mediated resistance to PI3K inhibitors in HER2-overexpressing breast cancer cells. Oncogene 2015 May 11; PMID:25961924
8. Amin DN, Campbell MR, Moasser MM. The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics. Sem Cell Dev Biol 2010; 21:944–950; http://dx.doi.org/10.1016/j.semcdb.2010.08.007
9. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huez O, Serra V, Majumder PK, Baselga J, Rosen N. AKT inhibition relieves suppression of receptor tyrosine kinase expression and activity. Cancer Cell 2011; 19:58–71; PMID:21215704; http://dx.doi.org/10.1016/j.ccr.2010.10.031
10. Singh A, Ye M, Bucur O, Zhu S, Tanya Santos M, Rabinovitz I, Wei W, Gao D, Hahn WC, Khosravi-Far R. Protein phosphatase 2A reactivates FOXO3a through a dynamic interplay with 14-3-3 and AKT. Mol Biol Cell 2010; 21:1140–1152; PMID:20110348; http://dx.doi.org/10.1091/mbc.E09-09-0795