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RAPID COMMUNICATION

Loss of the *emei* tumor suppressor promotes tumorigenesis via the JNK and Hippo pathway

Mutations in the *Ras* oncogene are the most frequently cancer alterations, occurring in more than 30% of all human cancers. The failures in the development of successful clinical inhibitors against Ras have made Ras as a "undruggable" target. The major reason is lack of a systematic understanding of oncogenic cooperation between *Ras* activation and mutation of related tumor suppressor genes.[1] The genetic techniques available in the *Drosophila melanogaster* allow analysis of the behavior of cells with distinct mutations, making this the ideal model organism to dissect oncogenic cooperation induced tumorigenesis.[2] The exact same mutation, e.g., Ras^{V12}, was mimicked in the *Drosophila*, which enables us to perform large scale genetic screens, aiming to unearth novel tumor suppressors that can synergistically enhance Ras^{V12}-related tumor growth.

Our lab has previously performed an ethyl methanesulfonate (EMS)-induced forward genetic screen on *Drosophila* chromosome 3L by utilizing the *ey-FLP* based mosaic analysis with a repressible cell marker (MARCM) technique. We identified Emei, the homolog of human TMEM161B (transmembrane protein 161B), as an endoplasmic reticulum (ER) localized protein that regulates Ca^{2+} homeostasis.[3] *TMEM161B* is located on 5q14, a region that is frequently deleted in a variety of neoplasms, such as ovarian and lung cancers.[4] The exact roles of TMEM161B in the cancer remain largely uncharacterized.

Our lab has established that the growth of $Ras^{V12}emei^{-/-}$ tumors involves the JNK and Hippo signaling pathways.[3] It has been unclear whether disruption of *emei* alone can activate JNK signaling and trigger JNK-dependent invasive cell migration. We specifically knocked down *emei* along the anterior-posterior (A/P) boundary of wing disc under the control of *patched* promoter (*ptc-Gal4*) and examined the expression of JNK target genes including *puckered* (*puc-lacZ*) and *Mmp1*. Depletion of *emei* significantly increased levels of *puc-lacZ* and *Mmp1* (Fig. 1A, C). A similar phenotype was observed using another wing disc related Gal4, namely *en-Gal4*, that exclusively drives gene expression in the posterior wing compartment (Fig. 1A).

Interestingly, knocking down of *emei* driven by *ptc-Gal4* also triggers cell death and generates a loss of anterior cross vein (ACV) phenotype in the adult wing (Fig. 1B). Inhibiting apoptosis by ectopically expressing the caspase inhibitor p35 did not rescue this phenotype (Fig. 1B), indicating that this ACV loss phenotype is most likely caspase activity independent. Blocking JNK signaling by expressing the JNK phosphatase Puc, bsk^{DN} (dominant-negative form of Drosophila *JNK*), or depleting the JNK kinase *hemipterous* (*hep*), all dramatically inhibited the loss-of-ACV phenotype (Fig. 1B). Therefore, these data demonstrate that Emei negatively regulates JNK signaling by genetically acting upstream of Hep.

In the *Drosophila* wing epithelia, loss of some tumor suppressor genes or oncogene activation along the A/P boundary produces an invasive migration phenotype, which has been extensively used to model cell invasion *in vivo*.[2] Knockdown of *emei* along the A/P boundary induces significant invasive migration and Mmp1 upregulation, both of which could be completely reversed by expressing bsk^{DN} (Fig. 1C). Taken together, these data suggest that loss of *emei* activates JNK signaling to induce oncogenic cell migration.

Next, we investigated the molecular mechanism of loss of *emei*-mediated JNK activation. To avoid the off-target effect of RNAi, further experiments were performed by an eye-specific source of Flippase (*ey-Flp*) using the MARCM system to generate *emei* mutant clones in eye discs. Indeed, *emei* mutant clones also showed strongly increased *puc-lacZ* levels (Fig. 1D). Among the wide palette of cellular events leading to JNK activation is *dTNF/eiger* (*egr*). Egr is the sole *Drosophila* member of the TNF superfamily and serve as a ligand for the *Drosophila* JNK pathway.[2] To identify *emei* mutant clones activates JNK activation through Egr signaling, we first asked whether Egr is upregulated in these clones. We found that Egr protein level did not change significantly in *emei* mutant clones or surrounding cells

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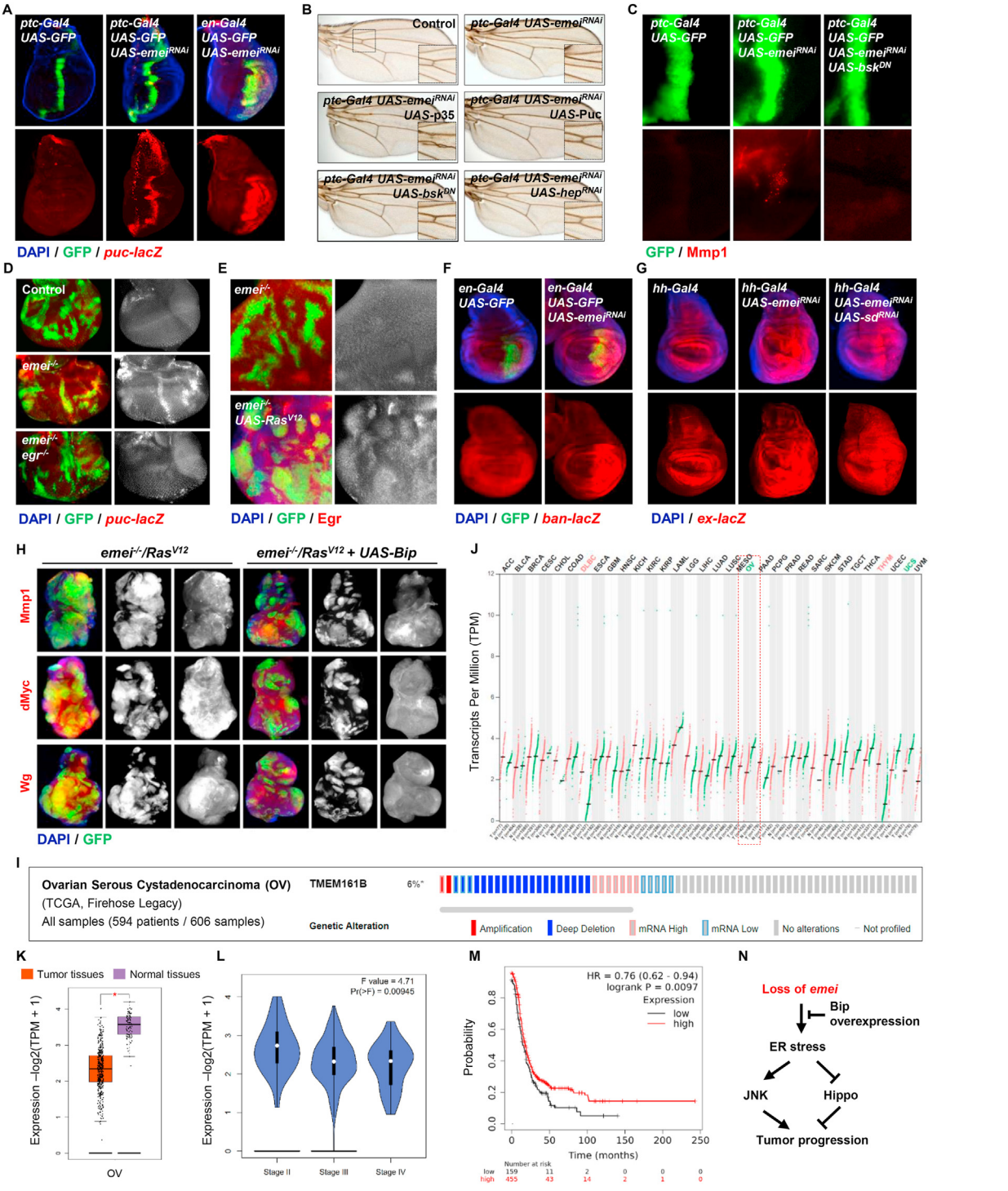

Figure 1 *Emei* regulates tumor progression via JNK and Hippo signaling. **(A)** Different genetic manipulations driven by *ptc-Gal4* or *en-Gal4*. Wing discs were immunostained with anti-β-Gal antibody and DAPI. **(B)** Adult wings of the indicated genotypes using *ptc-Gal4*. **(C)** Different genetic manipulations driven by *ptc-Gal4*. Wing discs were immunostained with anti-Mmp1 antibody. **(D, E)** Eye discs containing GFP-marked MARCM clones of the indicted genotypes and were immunostained with anti-β-Gal antibody or anti-Egr antibody. **(F, G)** Different genetic manipulations driven by *en-Gal4* or *hh-Gal4*. Wing discs were immunostained with anti-β-Gal antibody and DAPI. **(H)** Eye discs containing GFP-marked MARCM clones of the indicted genotypes and were immunostained with anti-Mmp1, anti-dMyc, or anti-Wg antibody. **(I)** Alteration frequency and oncoprint diagram of *TMEM161B* gene-alteration summary in patients with cancer (11,413) obtained from cBioPortal (The Cancer Genome Atlas, TGCA). **(J)** Pan-cancer transcription levels of *TMEM161B* gene in patients with cancer obtained from GEPIA 2. **(K, L)** *TMEM161B* expression is specially significantly lower in OV tumor samples compared to normal tissues and the lower levels of *TMEM161B* was existed in stage III and IV. **(M)** Low levels of *TMEM161B* expression correlated significantly with poor survival of patients. *P* values were determined by log-rank (Mante-Cox) test. **(N)** Proposed model of how Emei promotes tumorigenesis via the JNK and Hippo pathway.

(Fig. 1E), but the increased *puc-lacZ* levels in *emei* mutant clones were completely abolished in animals that were homozygous mutants for *egr* ($egr^{-/-}$) (Fig. 1D). These results suggested that Egr-JNK signaling may not originate within the *emei* mutant clones but instead comes from host environment. Interestingly, overexpression of oncogenic *Ras* (Ras^{V12}) in discrete $emei^{-/-}$ clones ($emei^{-/-}/Ras^{V12}$) also leads to up-regulation of Egr protein levels (Fig. 1E). This suggests that host environment-derived Egr signaling or *Ras* activation-highjacked Egr signaling is a tumor promoter in *Ras-emei* oncogenic cooperation.

we further dissected whether loss of *emei* alone can also inactivate Hippo signaling. We silenced *emei* and examined the expression of Yki target genes such as *ban*, *ex*. Knockdown of *emei* dramatically increased *ban-lacZ* (Fig. 1F) and *ex-lacZ* expression (Fig. 1G). Upon Hippo signaling perturbation, Yki translocates into the nucleus and forms a functional complex with transcription factor Scalloped (*sd*), which is a critical step in transcriptional regulation.[3] The upregulation of *ex-lacZ* by knocking down *emei* could be reversed by depletion of *sd* (Fig. 1G). Overall, these results suggest that *emei* negatively regulates expression of Yki target genes in the developing wing.

Emei is an ER localized transmembrane protein that is essential for ER Ca^{2+} storage, suggesting that its dysregulation may disrupt ER homeostasis. Bip is located mainly in the ER, which acts a molecular chaperone to orchestrate serval of endogenous cytoprotective mechanisms.[5] Thus, we hypothesized that ER stress attenuation through Bip overexpression could inhibit progression of $emei^{-/-}/Ras^{V12}$ tumors. As expected, Bip overexpression dramatically restrained the tumor growth phenotype (Fig. 1H). Moreover, ectopic expression of Bip significantly inhibited upregulation of JNK target gene *Mmp1*, as well as Yki target genes including *Myc* and *Wg* (Fig. 1H). Therefore, these findings imply that attenuation of ER stress may collectively regulate both JNK activation and Hippo signaling to suppresses *Ras-emei* cooperation induced tumorigenesis.

To explore whether TMEM161B is involved in human cancer progression, we assessed the roles of *TMEM161B* in pathogenesis using available online tools and bioinformatics analysis, and found that *TMEM161B* is located on 5q14, a region that is frequently deleted in several human cancers (especially in ovarian cancer (OV)[4] (Fig. 1I). Moreover, we found that *TMEM161B* expression is significantly lower in OV tumor samples, compared with normal tissues in the multi-cancer database (Fig. 1J). In addition, *TMEM161B* mRNA expression level was significantly correlated with tumor stages, and reduced expression of *TMEM161B* was observed across advanced stages, including stage III and IV (Fig. 1K, L). Consistently, low levels of *TMEM161B* expression are also correlated significantly with poor survival of OV patients (Fig. 1M).

Overall, we show that loss of *emei* alone activates JNK signaling and inhibits Hippo signaling that thus enhances tumor growth and stimulates invasive migration (Fig. 1N). Our findings here provide mechanistic insights into *emei* related signaling transduction and demonstrate that disruption of the emei-JNK-Hippo axis might bridge Ca^{2+} homeostasis with ER stress and cancer progression. Clinically, TMEM161B expression specially correlated with survival of patients with ovarian cancer. Together with the work described here, these data highlight the importance of a novel ER transmembrane protein in tumorigenesis.

Author contributions

X.M. conceived and supervised the study; S.Y. designed the experiments and wrote the manuscript; S.Y. performed experiments; S.Y. and Y.G. analyzed the data.

Conflict of interests

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.02.020.

References

1. Moore AR, Rosenberg SC, McCormick F, Malek S. RAS-targeted therapies: is the undruggable drugged? *Nat Rev Drug Discov*. 2020;19(8):533–552.
2. Tapon N. Modeling transformation and metastasis in Drosophila. *Cancer Cell*. 2003;4(5):333–335.
3. Ma X, Lu JY, Moraru A, et al. A novel regulator of ER Ca(2+) drives Hippo-mediated tumorigenesis. *Oncogene*. 2020;39(6):1378–1387.
4. Tavassoli M, Steingrimsdottir H, Pierce E, et al. Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by *TP53* mutation and identifies a tumour suppressor gene locus at 5q13.1-21. *Br J Cancer*. 1996;74(1):115–119.
5. Casas C. GRP78 at the centre of the stage in cancer and neuroprotection. *Front Neurosci*. 2017;11:177.

Shuai Yang [a,b,c], Yifan Guo [a,b,c], Xianjue Ma [a,b,c,*]

[a] *Key Laboratory of Growth Regulation and Translational Research of Zhejiang Province, School of Life Sciences, Westlake University, Hangzhou, Zhejiang 310024, China*
[b] *Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou, Zhejiang 310024, China*
[c] *Institute of Biology, Westlake Institute for Advanced Study, Hangzhou, Zhejiang 310024, China*

*Corresponding author. Key Laboratory of Growth Regulation and Translational Research of Zhejiang Province, School of Life Sciences, Westlake University, Hangzhou, Zhejiang 310024, China.
E-mail address: maxianjue@westlake.edu.cn (X. Ma)

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