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LETTER TO THE EDITOR

BAIAP2L1 enables cancer cell migration and facilitates phospho-Cofilin asymmetry localization in the border cells

Dear Editor,

Cancer invasion and metastasis are among the most clinically relevant cancer hallmarks. Identification of molecular pathways that contribute to cancer cell motility a crucial step in cancer invasion/metastasis is essential to understanding how cancer motility is regulated and may provide therapeutic targets for treating metastatic cancer. Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 (BAIAP2L1) is a novel oncoprotein whose high-leveled expressions have been correlated with advanced and metastatic stages of cancer [1–3]. We have previously uncovered BAIAP2L1 as a novel oncogene by integrative analysis of protein-protein interactome and somatic copy number alterations in human cancers [4]. Although in vivo xenograft model showed that BAIAP2L1 promoted tumor growth [4], however, the exact mechanism by which BAIAP2L1 facilitates tumorigenesis remains unclear.

We hypothesized that expressions of BAIAP2L1 were involved with metastatic/invasive phenotypes of cancer. To investigate, we selected the breast cancer cell line MCF7 which expressed an acceptable level of BAIAP2L1 mRNA (Supplementary Fig. S1). Detailed methods can be found in the Supplementary File. We depleted endogenous BAIAP2L1 in MCF7 by BAIAP2L1-specific short-hairpin RNAs (shRNAs) and measured cancer cell migration via wound-healing assay. We found that BAIAP2L1-specific shRNAs depleted 80% of BAIAP2L1 and reproducibly suppressed MCF7 migration (Figures 1A and 1B and Supplementary Fig. S2A, B). To study the role of the BAIAP2L1 gain-of-function, we tested MCF7 clones stably overexpressing BAIAP2L1. Overexpression of BAIAP2L1 in MCF7 (Supplementary Fig. S2C) significantly enhanced wound healing (Supplementary Fig. S2D-F). The results were further confirmed in cell line TFK1 (Supplementary Fig. S2G-N). To examine the role of BAIAP2L1 in the chemotaxis/directional invasion through the extracellular matrix, the transwell invasion assay was performed. BAIAP2L1 depletions significantly reduced MCF7 cell invasiveness (Figure 1C). We confirmed that the results were not influenced by the altered cell viability, apoptosis, or cell adherence by performing the MTT, apoptosis, and cellular adhesion assays, respectively (Supplementary Fig. S3A-C). Therefore, we concluded that expressions of BAIAP2L1 were correlated with the ability of cancer cells to migrate and invade.

To investigate how BAIAP2L1 contributes to cancer migration, we performed co-immunoprecipitation-mass spectrometry of the BAIAP2L1-containing protein complex in the MCF7 cells expressing 3 × FLAG-BAIAP2L1 (Supplementary Fig. S4A). Among the top 40 BAIAP2L1-interacting proteins were proteins in cell migration and/or wound healing, namely Aldolase A (ALDOA), Cofilin (CFL1), Capping actin protein of muscle Z-line subunit alpha 2 (CAPZA2) (Supplementary Fig. S4B, and Supplementary Table S1). The full lists of proteins identified in the sample (Supplementary Table S2) and control (Supplementary Table S3) are shown in the Supplementary Files. We noticed an enrichment of the filopodium-like protrusion (FLP) complex proteins, i.e. p21 (RAC1) activated kinase 2 (PAK2), Actin-related protein 2 (ARP2), cofilin, and Formin like 1 (FMNL1). We validated some of the interactors, namely ALDOA and cofilin, by immunoprecipitations: immunoblottings (Supplementary Fig. S4C). These data suggested that BAIAP2L1 is part of the proteins functioning in FLP formation, and migration (Supplementary Table S4).

Abbreviations: ALDOA, Aldolase A; ARP2, Actin-related protein 2; BAIAP2L1, Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1; CAPZA2, Capping actin protein of muscle Z-line subunit alpha 2; CFL1, Cofilin 1; DMFS, Distant metastasis-free survival; DOX, Doxycycline; F-actin, Filamentous actin; FLP, Filopodium-like protrusion; FMNL1, Formin like 1; HA, Human influenza hemagglutinin; HER-2, Human epidermal growth factor receptor 2; LIMK, LIM domain kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; OS, Overall survival; PAK2, p21 (RAC1) activated kinase 2; pCofilin, Ser3-phosphorylated cofilin; RWD, Relative wound density; SD, Standard deviation; Sh, Short hairpin; Sh-BAIAP2L1, BAIAP2L1-specific short hairpin RNA; Sh-ctrl, Non-specific short hairpin RNA; shRNAs, Short hairpin ribonucleic acids

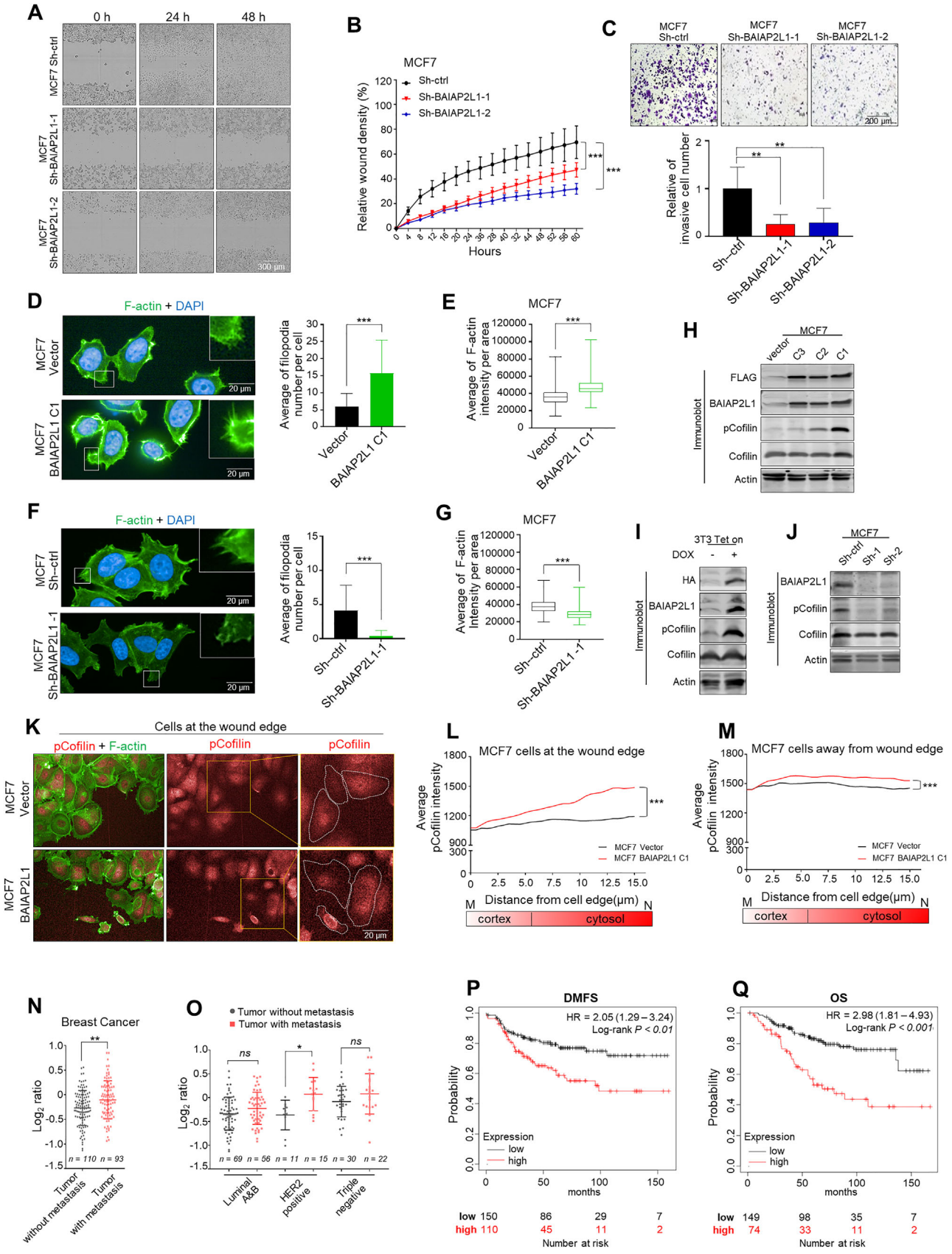

FIGURE 1 BAIAP2L1 is a key protein that controls cancer cell migration via pCofilin regulation

(A-B) Depletions of BAIAP2L1 delay cancer cell migration. (A) 10 × magnification wound healing images of MCF7 in control (MCF7 sh-ctrl) and BAIAP2L1-knockdown MCF7 cells (MCF7 Sh-BAIAP2L1-1 and MCF7 Sh-BAIAP2L1-2) are shown at 0, 24 and 48 hours after scratch. The

The FLP complex produces filopodia to determine the cell movement direction. We found a 3-fold increase in filopodia numbers accompanied by an upregulation of filamentous actin (F-actin) in the BAIAP2L1-overexpressing MCF7 cells (Figures 1D and 1E). Conversely, depletions of BAIAP2L1 in the MCF cells resulted in a 3-fold reduction of filopodia numbers and decreased F-actin formation (Figures 1F and 1G). In the BAIAP2L1-overexpressing cells, we found that F-actin formation was upregulated at the edge of the wound, specifically, at the cortex (Supplementary Fig. S5A-C). Importantly, the total level of actin was not altered by BAIAP2L1 expression (Supplementary Fig. S5D).

To direct the movement, polymerization of monomeric actins forms filopodia at the cortex of the migrating cells. Cofilin facilitates actin polymerization via its actin-severing activity providing free barbed ends for actin reorganization [5]. Overexpression of FLAG-BAIAP2L1 did not alter the expressions of cofilin (Figure 1H, and Supplementary Fig. S6A) but consistently increased the pool of the Ser3-phosphorylated cofilin (pCofilin) in the MCF7 clones (Figure 1H, and Supplementary Fig. S6A). To confirm that the increased pCofilin was not caused by a long-term adaptation of the BAIAP2L1-overexpressing clones, we established an inducible HA-BAIAP2L1-overexpressing mouse 3T3 cell line. We detected a significant upregulation of pCofilin, but not total cofilin, in the inducible HA-BAIAP2L1-overexpressing cells upon the doxycycline treatment (Fig. 1I, and Supplementary Fig. S6B), indicating that increased pCofilin was driven by the BAIAP2L1 induction. In contrast, pCofilin was suppressed in the BAIAP2L1-depleted cells (Figure 1J, and Supplementary Fig. S6C). From these results, we concluded that BAIAP2L1 levels regulate the pool of pCofilin in the cell.

Within the cell, activated cofilin accumulates at the cortex of the migrating cells, while inactivated cofilin localizes at the inner part of cytosol, where the activity of cofilin is suppressed by LIMK-dependent cofilin SER3 phosphorylation [6]. Relocalization of pCofilin from the cellular cortex to the cytosol enables the asymmetric distribution of active/inactive cofilin, allowing a reorganization of the actin filament. In agreement with this view, we found that in the migrating cells at the wound edge, expression of pCofilin gradually increased along the cortex to cytosol axis (Fig. 1K upper panels, 1L black line). Interestingly, we found that the overexpression of BAIAP2L1 significantly increased the cellular levels of pCofilin and potentiated the localization of pCofilin in the cytosol (Supplementary Fig. S7A, Figure 1K, lower panel, 1L red line). The same observations were not found in the cells located away from the wound edge (Figure 1M). Depletion of BAIAP2L1 reduced overall pCofilin expressions and attenuated the inward gradient of pCofilin to the background level (Supplementary Fig. S7B-D). From these data, we concluded that the expression of BAIAP2L1 is a key factor facilitating a spatial accumulation of pCofilin at the inner part of the migrating cell. To examine the clinical relevance of BAIAP2L1, we surveyed BAIAP2L1 expressions in breast cancer from the Human Cancer Metastasis Database

cells were pre-treated with mitomycin C to minimize the impact of cell proliferation. (B) Relative wound density (RWD) over 60 hours of MCF7 cells expressing Sh-ctrl, sh-BAIAP2L1-1, or sh-BAIAP2L1-2, 100% indicates a completely closed gap. (C) Depletions of BAIAP2L1 suppress cancer cell invasion. Transwell Matrigel invasion assay of the control MCF7 cell line (MCF7 Sh-ctrl) and the knockdowned MCF7 cell lines (Sh-BAIAP2L1-1 and Sh-BAIAP2L1-2) (shown in 10 × magnification). The relative numbers of invasive cells are shown in the bar graphs. The data are represented as mean ± SD. (D-E) BAIAP2L1 overexpression promotes F-actin formation. (D) Immunofluorescence staining of F-actin (63 ×) of the control MCF7 cells expressing empty vector (Vector) and the BAIAP2L1-overexpressing MFC7 cells (BAIAP2L1 C1). (F-actin; green, and nuclear; blue). Boxed insets are the zoom-in of selected areas, highlighting the filopodia as thin protrusions from the cell surface with a length of 2 μm or longer. Means ± SD of filopodium number per cell are shown in bars. (E) The average F-actin intensity per area from cells in (D). (F-G) BAIAP2L1 depletion reduces F-actin formation. (F) Immunofluorescence staining of F-actin (63 ×) of the control MCF7 cells expressing non-specific shRNA (Sh-ctrl) and the BAIAP2L1-expressing shBAIAP2L1 (F-actin; green, and nuclear; blue). Means ± SD of filopodium number per cell are shown in bars. (G) The average F-actin intensity per area from cells in (F). (H) BAIAP2L1 expressions increase the expression of pCofilin in MCF7 cells. Expressions of indicated proteins in the BAIAP2L1-overexpressing MCF7 clones (C1, 2, 3,) or control MCF7 (Vector). (I) pCofilin expression is induced by the inducible BAIAP2L1 expression. Expressions of indicated proteins in the inducible BAIAP2L1-expressing 3T3 cells with or without doxycycline (DOX) treatment. (J) Depletions of BAIAP2L1 downregulate pCofilin. Expressions of indicated proteins in the BAIAP2L1-depleted MCF7 cell lines (Sh-1, and Sh-2) compared to control MCF7 cells (Sh-ctrl). (K-M) BAIAP2L1 expression promotes the spatial distribution of pCofilin toward the inner part of the cells at the wound edge. (K) Immunofluorescent images (20 ×) of the cells at the wound edge. Expressions of pCofilin (red) and F-actin (green) in the BAIAP2L1-expressing MCF7 cells (BAIAP2L1 C1), and control cells (Vector). (L) Average intensities of intracellular pCofilin from (K) and from cells located away from the wound edge (M). (N-O) Expressions of BAIAP2L1 are associated with metastatic potential in breast cancer. (N) Expressions of BAIAP2L1 in early-stage patients without metastasis *vs.* those who eventually developed metastasis. (O) data from (N) divided into breast cancer subtypes. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$; *ns* = not statistically significant. (P-Q) Kaplan-Meier curves revealed the poorer overall survival (OS) and distant metastasis-free survival (DMFS) for HER2-positive breast cancer patients with high BAIAP2L1 expression, compared to the patients with low BAIAP2L1 expression

(Supplementary File). We found that high BAIAP2L1 expression was significantly associated with metastatic primary breast cancer (Figure 1N) and was the most significantly correlated with the HER2-positive subtype (Figure 1O). Kaplan–Meier survival curves indicated that the distant metastasis-free survival (DMFS) ($P < 0.005$) and overall survival (OS) ($P < 0.001$) were significantly poorer in the high BAIAP2L1 HER2-positive breast cancer patients (Figure 1P, Q). Therefore, our data suggest that BAIAP2L1 is associated with metastatic breast cancer and may be used as an independent prognostic indicator for the clinical outcomes of patients with breast cancer.

In normal cells, BAIAP2L1 can modulate clusters of short actin bundles during cell movement [7]. We demonstrated that the gain-of-function of BAIAP2L1 could promote cancer cell migration/invasion. This notion is backed up by our findings from the clinical databases (Figure 1N-Q). We also uncovered a novel relationship between BAIAP2L1 and cofilin, a key protein that initiates early steps in the motility cycle. Similar to that of BAIAP2L1, misregulations of the cofilin pathway have been recently documented in invasive cancer [8]. Upregulations of cofilin or phospho-Ser3-cofilin, without any control for the distribution, disrupted the cell migration rather than promoted it [9–11]. From our results, we concluded that BAIAP2L1 is a protein that regulates the distribution of cofilin and phospho-Ser3-cofilin to create a directional movement, signifying BAIAP2L1 as a key protein in cancer cell migration. The mechanism by which BAIAP2L1 orchestrates the complex cofilin regulation requires further investigation. Altogether these data support the BAIAP2L1 pathway as a novel therapeutic target for highly aggressive cancer.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable. This study used only the public anonymized data available from public domains which are completely and anonymized. The data do not contain any personal data and do not allow tracking back to the personal data.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article.

COMPETING INTERESTS

The authors declare no competing or financial interests.

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AUTHOR CONTRIBUTIONS

N.P. and S.J. designed this study. N.P. performed most of the experiments with the help of N.W., S.P., W.P., T.P., W.K. and analyzed the data with N.P. S.S. provided executive support in the data analysis. N.P. and S.J. wrote the manuscript. S.J. provided the overall direction of the project. All authors reviewed the manuscript critically and approved the content. All authors and corresponding author of this work bear full responsibility of all aspects of this work.

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