Edil3/Del-1-induced phosphorylation of AMPKβ facilitates progression and correlates with poor prognosis in triple-negative breast cancer

Soo Jung Lee[1*], Seol-Hwa Jeong[2*], Jeeyeon Lee[3], In Hee Lee[1], Byeongju Kang[3], Joon Suk Moon[3], Ho Yong Park[3] Ji-Young Park[4], Nora Jee-Young Park[4], Eun Ae Kim[5], Jieun Kang[5] & Yee Soo Chae[1]

[1]Department of Oncology/Hematology, Kyungpook National University Chilgok Hospital, School of Medicine, Kyungpook National University, Daegu, Republic of Korea. [2]Cell & Matrix Research Institute, Kyungpook National University, Daegu, Republic of Korea.
[3]Breast & Thyroid Surgery, Kyungpook National University Chilgok Hospital, School of Medicine, Kyungpook National University, Daegu, Republic of Korea. [4]Department of Pathology, Kyungpook National University Chilgok Hospital, School of Medicine, Kyungpook National University, Daegu, Republic of Korea
[5]Kyungpook National University Chilgok Hospital, Daegu, Republic of Korea.

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Background

Triple-negative breast cancer (TNBC) is a clinically aggressive subtype with limited therapeutic options. Developmental endothelial locus-1 (EDIL3/Del-1), a secreted glycoprotein found in extracellular vesicles, has been implicated in TNBC, but its mechanism of action remains unclear. AMP-activated protein kinase (AMPK) is a key metabolic sensor, and its β subunit (AMPKβ) is often amplified in tumors. This study investigates the role of Del-1–mediated phosphorylation of AMPKβ in TNBC progression and patient prognosis.

Methods and Materials

Expression of Del-1 and AMPK subunits was evaluated in TNBC cell lines using RT-PCR and Western blot. Functional assays including MTT, MTS, BrdU, and Matrigel invasion assays were conducted in CRISPR/Cas9 Del-1 knockout cells, the treatment of AMPKβ activator and phospho-mimetic AMPKβ-over-expressed cells. Tumoral AMPKβ expression was assessed via immunohistochemistry on tissue microarrays from 111 early-stage TNBC patients. Publicly available survival data (KM Plotter) were also analyzed.

Results

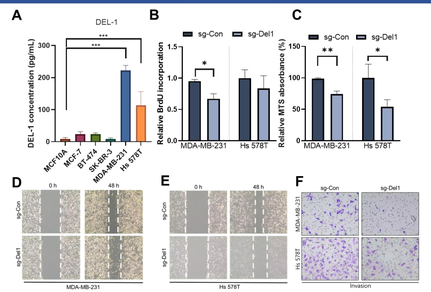


Fig. 1. DEL-1 drives progression in mesenchymal-like TNBC cells. (A) Protein levels of DEL-1 were determined by ELISA across the indicated cell types.(B-C) Proliferation and viability were assessed using BrdU incorporation and MTS assays, respectively, in sgDel1 mesenchymal-like TNBC cells. (D-F) The migratory and invasive capacities of sgDel1 mesenchymal-like TNBC cells were evaluated via migration and invasion assays.

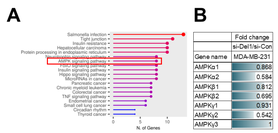


Fig. 2. AMPK pathway suppression upon DEL-1 knockdown in breast cancer cells. (A) Gene enrichment analysis highlights a positive correlation between DEL-1 and AMPK signaling. (B) Differentially expressed genes were identified in DEL-1-silenced MDA-MB-231 cell line.

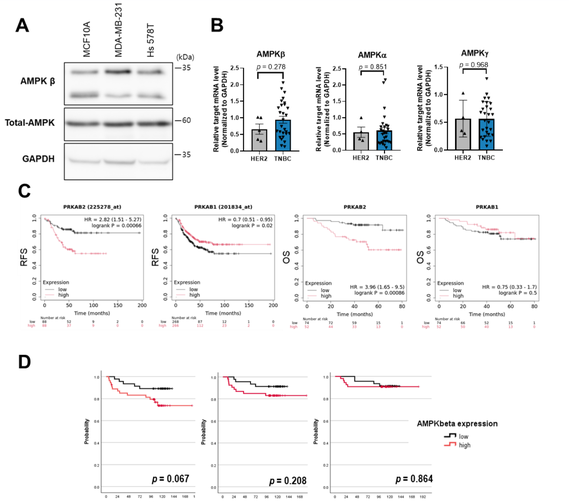


Fig. 3. Elevated AMPKβ expression in mesenchymal-like TNBC cells correlates with an increasing trend in patient tumor samples. (A) We assessed the protein expression of AMPKβ, AMPKα, and AMPKγ by western blot analysis. (B) Quantitative real-time PCR was performed to determine the mRNA levels of AMPK subunits in patient tumor tissues (C) Kaplan–Meier statistics demonstrate the association of AMPKβ mRNA expression with overall survival and RFS in TNBC patients (n=126). (D) Survival curves based on AMPKβ expression in a cohort of 100 patients (n = 100). Disease-free survival (DFS, p = 0.067), distant disease-free survival (DDFS, p = 0.208), and overall survival (OS, p = 0.964) are shown.

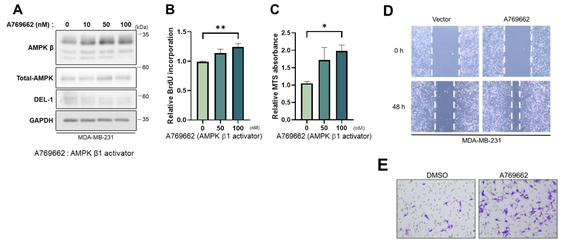


Fig. 4. Activation of AMPKβ through pharmacological intervention drives breast cancer cell proliferation and invasiveness. (A) Western blot analysis was performed to evaluate the protein levels of AMPKβ, total AMPK, and DEL-1 in MDA-MB-231 cells exposed to escalating concentrations of the AMPKβ activator A769662 (0–100 nM) for 24 h. (B) BrdU assays indicated a significant enhancement in proliferation following pharmacological AMPKβ activation. (C) MTS assays demonstrated an increase in cell viability in MDA-MB-231 cells upon treatment with the AMPKβ activator. (D) Migration assays evidenced an elevation in cell motility after treatment with the AMPKβ activator. (E) Increased invasiveness of MDA-MB-231 cells, treated with 100 nM AMPKβ activator for 48 h, is depicted by representative images from transwell invasion assays.

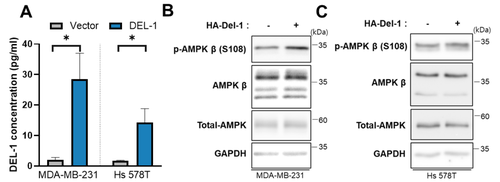


Fig. 5. Overexpression of DEL-1 upregulates phosphorylated AMPKβ and contributes to TNBC cell progression. (A) DEL-1 protein levels were confirmed by ELISA after DEL-1 overexpression in mesenchymal-like TNBC cell lines. (B, C) Western blot analysis revealed the expression patterns of p-AMPKβ(S108) and AMPKβ in MDA-MB-231 (B) and Hs 578T (C) cells overexpressing DEL-1. (D) BrdU assays indicated elevated proliferation rates in MDA-MB-231 and Hs 578T cells with DEL-1 overexpression after 48 hours. (E) MTS assays assessed the effect of DEL-1 overexpression on cellular proliferation. (F, G) Wound healing assays provided representative micrographs and quantified increased wound closure in DEL-1-overexpressing MDA-MB-231 (F) and Hs 578T (G) cells. (H) Transwell invasion assays, utilizing Matrigel-coated inserts, yielded representative images and quantified the enhanced invasiveness of DEL-1-overexpressing cells.

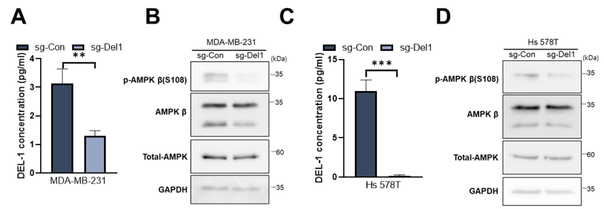


Fig. 6. DEL-1 knockdown significantly diminishes AMPKβ expression. (A, C) ELISA confirmed the DEL-1 protein levels of CRISPR-Cas9-mediated Del1 knockout MDA-MB-231(A) and Hs 578T cells(C). (B, D) Western blot analysis illustrates the expression of related proteins in MDA-MB-231(B) and Hs 578T(D) cells with CRISPR-Cas9-mediated Del1 knockout.

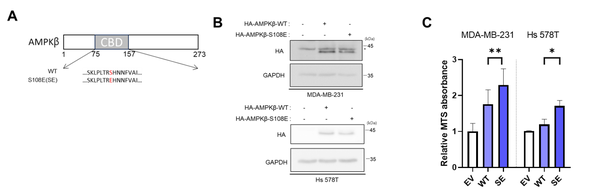


Fig. 7. Ser108 phosphorylation of AMPKβ by DEL-1 is indispensable for TNBC metastatic potential. (A) Schematic diagram indicates the genetic alteration of phosphorylation site. (B) Western blot analysis illustrates changes in indicated protein levels upon AMPKβ Wild-type (WT) and Phosphor-mimetic form (SE) in MDA-MB-231 and Hs 578T cells. (C) MTS survival assays show the effects of AMPKβ WT and SE overexpression in MDA-MB-231 and Hs 578T cells.

Conclusions

High AMPKβ expression in TNBC patient samples correlates with shorter distant disease-free and overall survival, underscoring its prognostic value. Complementing these clinical data, Del-1–mediated Ser108 phosphorylation of AMPKβ in mesenchymal stem-like TNBC cells promotes aggressive phenotypes, establishing AMPKβ as a promising biomarker and therapeutic target..

Acknowledgement

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Contact

Seol-Hwa Jeong
Cell & Matrix Research Institute, Kyungpook National University, Daegu, Republic of Korea
Email: jseolh@gmail.com

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