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DOCK1 inhibition suppresses cancer cell invasion and macropinocytosis induced by self-activating Rac1^{P29S} mutation

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ABSTRACT

Rac1 is a member of the Rho family of small GTPases that regulates cytoskeletal reorganization, membrane polarization, cell migration and proliferation. Recently, a self-activating mutation of Rac1, Rac1^{P29S}, has been identified as a recurrent somatic mutation frequently found in sun-exposed melanomas, which possesses increased inherent GDP/GTP exchange activity and cell transforming ability. However, the role of cellular Rac1-interacting proteins in the transforming potential of Rac1^{P29S} remains unclear. We found that the catalytic domain of DOCK1, a Rac-specific guanine nucleotide exchange factor (GEF) implicated in malignancy of a variety of cancers, can greatly accelerate the GDP/GTP exchange of Rac1^{P29S}. Enforced expression of Rac1^{P29S} induced matrix invasion and macropinocytosis in wild-type (WT) mouse embryonic fibroblasts (MEFs), but not in DOCK1-deficient MEFs. Consistently, a selective inhibitor of DOCK1 that blocks its GEF function suppressed the invasion and macropinocytosis in WT MEFs expressing Rac1^{P29S}. Human melanoma IGR-1 and breast cancer MDA-MB-157 cells harbor Rac1^{P29S} mutation and express DOCK1 endogenously. Genetic inactivation and pharmacological inhibition of DOCK1 suppressed their invasion and macropinocytosis. Taken together, these results indicate that DOCK1 is a critical regulator of the malignant phenotypes induced by Rac1^{P29S}, and suggest that targeting DOCK1 might be an effective approach to treat cancers associated with Rac1^{P29S} mutation.

1. Introduction

Rac1, which was originally discovered as Ras-related C3 botulinum toxin substrate 1 [1], is a member of the Rho family of small guanosine triphosphatases (GTPases) and functions as a molecular switch cycling between a GDP-bound inactive state and a GTP-bound active state [2,3]. Conversions to the active state and to the inactive state occur via GDP/GTP exchange and hydrolysis of GTP to GDP, respectively. Normally, Rac1 exists in the cells as a GDP-bound inactive state due to its marginal intrinsic GDP/GTP exchange activity and by the functions of GTPase-activating proteins (GAPs). Thus, activation of Rac1 requires the function of proteins called guanine nucleotide exchange factors (GEFs) that facilitate the nucleotide exchange. Once activated, Rac1 associates with a variety of downstream effectors and controls fundamental cellular processes including morphogenesis, migration, and proliferation through the regulation of cytoskeletal reorganization, membrane dynamics, and gene expression [2,3]. Aberrant expression and dysregulation of the activity of Rac1 have been linked to a variety of human cancers [4,5]. In cancer cells, activation of Rac1 leads to the

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formation of highly dynamic, actin-rich membrane structures such as invadopodia and circular dorsal ruffles that are implicated in three-dimensional migration, matrix invasion, and macropinocytosis [6–8]. Three-dimensional migration and matrix invasion are the basal mechanism of metastasis. Macropinocytosis is an endocytic process that internalizes a large portion of plasma membrane as well as extracellular fluid and its contents [8,9]. In Ras-transformed cells, Rac1-mediated macropinocytosis is exploited to feed on extracellular protein and supply amino acids for their sustained survival [10,11]. Thus, the invasion and macropinocytic activities facilitated by Rac1 contribute to tumorigenic and metastatic phenotypes of cancer cells.

Besides overexpression and alternative splicing [4,5], cancer-associated mutations of Rac1 have not been found frequently until recent, large genomic sequencing studies showing several gain-of-function mutations of Rac1 in human cancers [12–14]. Among the mutations, a hot spot C > T mutation in the RAC1 gene that changes proline at codon 29 to serine ($Rac1^{P29S}$) is a recurrent somatic mutation found in 9% of sun-exposed malignant melanomas, the third most frequent activating mutation in such melanomas after those of BRAF and NRAS [12]. $Rac1^{P29S}$ is shown biochemically to be self-activated due to substantially increased inherent GDP/GTP exchange [14,15]. Expression of $Rac1^{P29S}$ induces membrane ruffles, cell migration, proliferation, anchorage-independent growth, and gene expression in various cells [13–15]. So far, however, the role of cellular Rac1-interacting proteins in the transforming potential of $Rac1^{P29S}$ remains to be explored.

Two distinct families of Rac GEFs, the Dbl family and DOCK family, are currently known [16,17]. The DOCK family proteins are evolutionarily conserved, Rac/Cdc42 specific GEFs containing the lipid-binding DHR-1 (DOCK homology region-1) and the catalytic DHR-2 domains [17]. DOCK1 functions as a Rac specific GEF and regulates phagocytosis, myoblast fusion, and cell migration [17]. DOCK1 is critical for circular dorsal ruffle formation [18]. Aberrant expression and activity of DOCK1 is associated with malignant phenotypes in a variety of cancers [19–22]. Several lines of evidence indicate that DOCK1 regulates invasion and metastasis by acting downstream of receptor tyrosine kinases in glioblastoma and breast cancer cells [20–22]. Moreover, our recent study revealed that DOCK1 is a critical regulator of Rac activation that promotes invasion and macropinocytosis in Ras-driven cancer cells [23]. In this study, we found that DOCK1 can promote the GDP/GTP exchange of $Rac1^{P29S}$, and is essential for $Rac1^{P29S}$-induced invasion and macropinocytosis in mouse embryonic fibroblasts. Moreover, we show by genetic inactivation and pharmacological inhibition that DOCK1 inhibition can suppress the invasion and macropinocytosis in human cancer cells harboring $Rac1^{P29S}$ mutation.

2. Materials and methods

2.1. In vitro nucleotide exchange assays

Recombinant His-SUMO-tagged DOCK1 DHR-2 domain and GST-fused Rac1 proteins were prepared as described previously [23,24]. The assays consisted of GST-Rac1 (10 μM), His-SUMO-DOCK1 DHR-2 domain (0.05 μM), and Bodipy-FL-GTP (2.4 μM; G12411, Invitrogen) in the reaction buffer: 20 mM MES-NaOH, 150 mM NaCl, 1 mM $MgCl_2$, and 20 μM GDP, pH 7.0. GST-Rac1 was loaded with GDP by incubating with the reaction buffer on ice for 30 min, then mixed with Bodipy-FL-GTP and allowed to equilibrate at 30 °C for 2 min. The reaction was initiated by adding His-SUMO-DOCK1 DHR-2 domain (50 μl) to the GDP-loaded GST-Rac1/Bodipy-FL-GTP mixture (100 μl) in a final volume of 150 μl and incubating at 30 °C. The change in the fluorescence (Excitation: 488 nm/Emission: 514 nm) was recorded for 15 min with 15-s intervals using the Perkin Elmer EnSpire multimodal plate reader. Data were fitted with the curve fitting function of GraphPad Prism (GraphPad Software) to calculate the initial slope during the first 10 s (RFU/s: relative fluorescent unit per second).

2.2. Cell preparation and culture

Wild-type (WT) and DOCK1-deficient ($DOCK1^{-/-}$) MEFs were immortalized and infected with recombinant retroviruses as described previously [23], using retrovirus vectors pMX-IRES-GFP encoding N-terminally HA-tagged $Rac1^{WT}$ and $Rac1^{P29S}$. Clones with comparable expression of HA-tagged Rac1 proteins were selected for functional analyses. A human skin malignant melanoma cell line IGR-1 was purchased from Cell Lines Service (#300219). DOCK1-deficient (DOCK1-KO) IGR-1 was generated by the CRISPR/Cas9 system in combination with gene targeting by homologous recombination as previously described [23]. DOCK1-KO clones (#1 and #2) with different target guide sequences (target 1 and 2 [23], respectively) were selected. A human breast cancer cell line MDA-MB-157 was purchased from American Type Culture Collection. Cells were cultured in DMEM medium (Wako Pure Chemical Industries) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Nichirei Bioscience), 100 U/ml penicillin (Life Technologies), and 100 μg/ml streptomycin (Life Technologies).

2.3. Immunoblotting

Total cell lysates were prepared as described previously [23], and analyzed by immunoblotting with the following primary antibodies: rabbit anti-DOCK1 (#C4C12, Cell Signaling Technology, 1:1000 dilution), mouse anti-Rac1 (#23A8, Millipore, 1:3000), rabbit anti-HA-tag (#C29F4, Cell Signaling Technology, 1:2000 dilution), and anti-β-actin (I-19, Santa Cruz, 1:1000 dilution), and secondary antibodies conjugated with horse radish peroxidase (HRP): anti-rabbit immunoglobulin (IgG) (sc-2004, Santa Cruz, 1:2000 dilution), anti-mouse IgG (sc-2005, Santa Cruz, 1:2000 dilution), and anti-goat IgG (sc-2020, Santa Cruz, 1:2000 dilution). Twenty μg protein of total cell lysate per lane was used for Rac1 blot, and 50 μg protein for the others.

2.4. Invasion assays

Matrix invasion activity was measured by using the BioCoat matrigel invasion chambers (Corning, #354480). Briefly, cells (2.5×10^4 cells for MEFs, and 5×10^4 cells for MDA-MB-157) were suspended in serum-free medium (300 μl) and loaded onto the upper chamber with an 8 μm pore size membrane coated with a thin layer of Matrigel basement membrane matrix, which was placed in a 24-well plate containing DMEM supplemented with 5% FBS (MEFs) or 20% FBS (MDA-MB-157) (500 μl). When applied, cells were incubated with mediums supplemented with TBOPP (final concentrations of 6.25–12.5 μM) or DMSO (vehicle control, final 0.2%). After incubation for 22 h (MEFs) or 48 h (MDA-MB-157) at 37 °C, cells on the bottom side of the upper chamber membrane (invaded cells) were stained with a modified Giemsa method using the Diff-Quick kit (Sysmex), and then analyzed by an Axio Lab.A1 light microscope (Carl Zeiss). For each sample, the average number of invaded cells from four separate fields was determined.

2.5. Macropinocytosis assays

Macropinocytic activity was assessed by measuring the uptake of fluorescently labeled dextran [23]. Briefly, cells were seeded on fibronectin-coated glass bottom dishes (Matsunami Glass) for

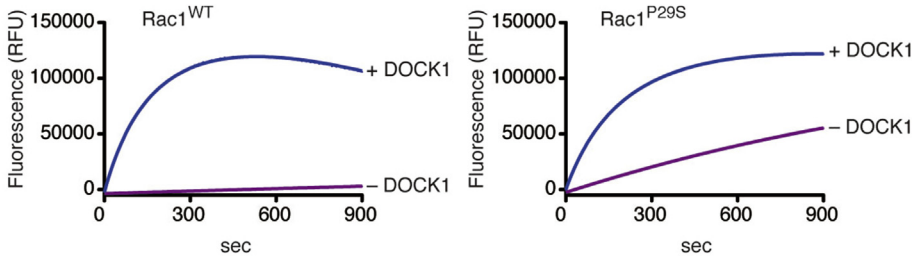

Fig. 1. DOCK1 promotes GDP/GTP exchange of Rac1^{P29S}.
Time course of nucleotide exchange assays. Conditions: GST-Rac1^{WT} or GST- Rac1^{P29S} (10 μM) was incubated with Bodipy-FL-GTP (2.4 μM) in the reaction buffer (20 mM MES-NaOH, 150 mM NaCl, 1 mM $MgCl_2$, and 20 μM GDP, pH 7.0) in the absence (− DOCK1) or presence (+DOCK1) of His-SUMO-DOCK1 DHR-2 domain (0.05 μM). RFU: relative fluorescence unit. Representative results of five independent experiments.

24–48 h. After serum starvation for 18 h (MEFs) or 14 h (IGR-1 and MDA-MB-157), cells were incubated for 1 h (MEFs) or 3 h (IGR-1 and MDA-MB-157) at 37 °C with DMEM containing 10% FBS and tetramethyl-rhodamine (TMR)-labeled dextran (D-1818, Invitrogen, molecular weight 70,000) at 0.25 mg/ml. When applied, cells were cultured in the mediums supplemented with TBOPP (final concentrations of 6.25–12.5 μM) or DMSO (vehicle control, final 0.2%) from the last hour of serum-starvation through the entire period of incubation with TMR-dextran. Then, cells were rinsed three times with ice-cold phosphate buffered saline (PBS)

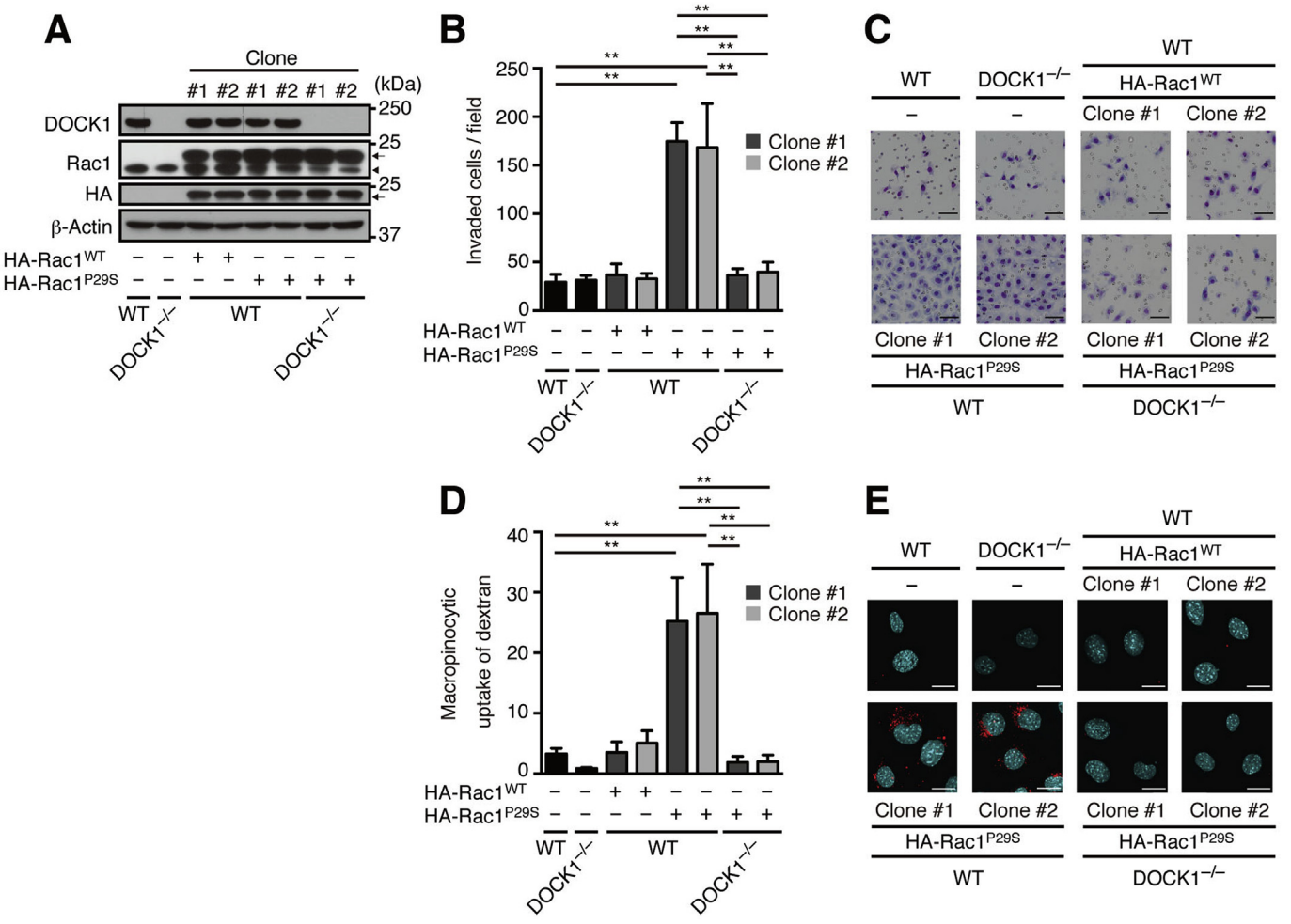

Fig. 2. DOCK1 is essential for Rac1^{P29S}-induced invasion and macropinocytosis in MEFs.
(A) Immunoblots showing the expression of DOCK1, Rac1, HA-tagged Rac1, and β-actin in WT and DOCK1$^{-/-}$ MEFs, and their clones expressing HA-tagged Rac1^{WT} or Rac1^{P29S}. Positions of HA-tagged Rac1 (arrows) and endogenous Rac1 (arrowhead) are indicated.
(B) Comparison of invasion activity among WT and DOCK1$^{-/-}$ MEFs, and their clones expressing HA-tagged Rac1^{WT} or Rac1^{P29S}. Data are means ± SD (standard deviation) of five independent experiments. **$p < 0.01$ (unpaired two-tailed Student's t-test).
(C) Representative images of invaded cells. Scale bar, 50 μm.
(D) Comparison of macropinocytic activity among WT and DOCK1$^{-/-}$ MEFs, and their clones expressing HA-tagged Rac1^{WT} or Rac1^{P29S}. Data are means ± SD of five independent experiments. **$p < 0.01$ (unpaired two-tailed Student's t-test). (E) Representative images. Scale bar, 20 μm.

and immediately fixed with 4% paraformaldehyde before nuclear staining with 4′,6′-diamidino-2-phenylindole (DAPI). Samples were analyzed by a laser scanning confocal microscope (Fluoview FV3000, Olympus).

2.6. Statistical analyses

Data were first examined by the Kolmogorov-Smirnov test for Gaussian distribution using the GraphPad Prism (GraphPad Software). Parametric data were analyzed by unpaired two-tailed Student's t-test, and non-parametric data were analyzed by Mann-Whitney *U* test to assess significant difference between two groups ($^{**}p < 0.01$).

3. Results

3.1. DOCK1 promotes GDP/GTP exchange of $Rac1^{P29S}$

To test whether DOCK1 can influence the activity of self-activating $Rac1^{P29S}$, we first examined the effect of DOCK1 DHR-2 domain on GDP/GTP exchange of $Rac1^{P29S}$ by using in vitro nucleotide exchange assays, in which the fluorescence intensity of Bodipy-FL-GTP, a fluorescent-analog of GTP, increases upon loading onto Rac1 protein. As previously reported [14,15], $Rac1^{P29S}$ exhibited significantly increased inherent GDP/GTP exchange activity in the presence of Mg^{2+} compared to wild-type Rac1 ($Rac1^{WT}$) (Fig. 1, the initial exchange rate of 85.0 ± 4.0 vs. 8.6 ± 1.5 RFU/sec, respectively). The addition of DOCK1 DHR-2 domain, the catalytic domain of DOCK1, greatly accelerated the GDP/GTP exchange reaction of $Rac1^{P29S}$ (606.1 ± 89.3 RFU/sec) as well as that of $Rac1^{WT}$ (971.1 ± 164.6 RFU/sec). The results indicate that DOCK1 has the ability to promote the GDP/GTP exchange of $Rac1^{P29S}$ directly.

3.2. DOCK1 is essential for transformation of MEFs by $Rac1^{P29S}$

To examine the role of DOCK1 in cellular transformation by $Rac1^{P29S}$, we introduced the expression of $Rac1^{P29S}$ or $Rac1^{WT}$ into immortalized wild-type (WT) and DOCK1-deficient ($DOCK1^{-/-}$) mouse embryonic fibroblasts (MEFs) via retrovirus infections, and selected their clones with comparable expression levels of HA-tagged $Rac1^{P29S}$ and $Rac1^{WT}$ (Fig. 2A). We found that the expression of $Rac1^{P29S}$, but not $Rac1^{WT}$, induced substantial matrix invasion and macropinocytic activities, typical cancerous phenotypes, in WT MEFs (Fig. 2B–E). While WT MEFs and $DOCK1^{-/-}$ MEFs barely exhibited invasion activity, WT MEFs expressing $Rac1^{P29S}$ exhibited significantly augmented invasion activity (Fig. 2B and C; 5.8-fold increase in average compared to WT MEFs). In striking contrast, $DOCK1^{-/-}$ MEFs expressing $Rac1^{P29S}$ did not show significant invasion. Similar results were obtained for macropinocytosis; WT MEFs expressing $Rac1^{P29S}$ showed enhanced macropinocytic activity (7.9-fold increase in average compared to WT MEFs) whereas $DOCK1^{-/-}$ MEFs expressing $Rac1^{P29S}$ did not show significant macropinocytic activity (Fig. 2D and E). These results indicate that DOCK1 is essential for cancerous transformation of MEFs by $Rac1^{P29S}$.

3.3. Pharmacological inhibition of DOCK1 suppresses invasion and macropinocytosis induced by $Rac1^{P29S}$ in MEFs

To further assess the role of DOCK1 in $Rac1^{P29S}$-induced cellular transformation, we employed our recently developed DOCK1-selective inhibitor, TBOPP (1-(2-(3'-(trifluoromethyl)-[1,1′-biphenyl]-4-yl)-2-oxoethyl)-5-pyrrolidinylsulfonyl-2(1H)-pyridone) [23]. TBOPP is a cell-active inhibitor that can bind to the DHR-2 domain of DOCK1 with low μM affinity and blocks its interaction with Rac1, and thereby inhibits its GEF activity without affecting the GEF activity of the Dbl family, Tiam1 and Trio [23]. As shown in Fig. 3A, treatment with TBOPP markedly suppressed the invasion in WT MEFs expressing $Rac1^{P29S}$ with the maximal inhibition at 12.5 μM to the level comparable to that of WT MEFs or $DOCK1^{-/-}$ MEFs. Consistently, the enhanced macropinocytosis in WT MEFs expressing $Rac1^{P29S}$ was suppressed by TBOPP treatment with similar efficacy to the level comparable to that of $DOCK1^{-/-}$ MEFs (Fig. 3B). These results indicate that the GEF activity of DOCK1 is critical for the transformation of MEFs by $Rac1^{P29S}$, and further demonstrate that pharmacological inhibition of DOCK1 can suppress cancerous transformation by $Rac1^{P29S}$.

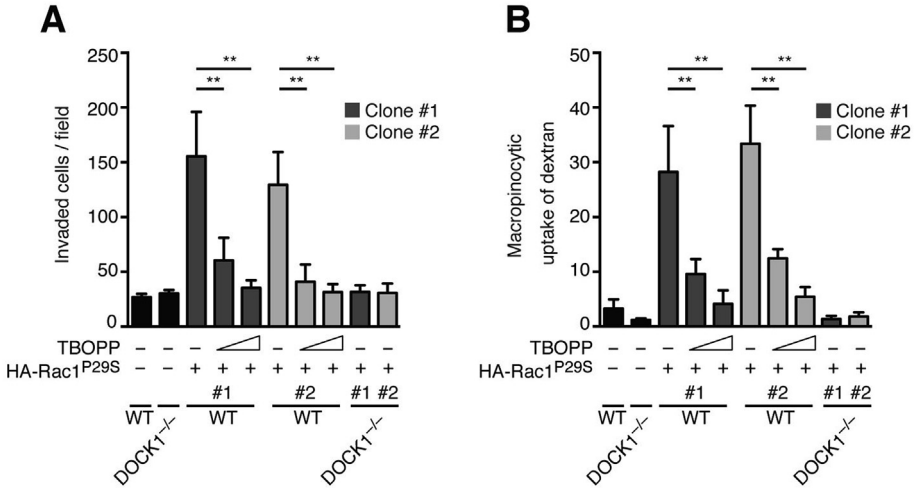

Fig. 3. Pharmacological inhibition of DOCK1 suppresses invasion and macropinocytosis in WT MEFs expressing $Rac1^{P29S}$.
(A) Effect of TBOPP on the invasion of WT MEFs expressing $Rac1^{P29S}$. Invasion activity of each clone treated with TBOPP (6.25, and 12.5 μM) or DMSO (−, vehicle control) was compared. Data are means ± SD of five independent experiments. $^{**}p < 0.01$ (unpaired two-tailed Student's t-test).
(B) Effect of TBOPP on the macropinocytic activity of WT MEFs expressing $Rac1^{P29S}$. Macropinocytic activity in each clone treated with TBOPP (6.25, and 12.5 μM) or DMSO (−, vehicle control) was compared. Data are means ± SD of five independent experiments. $^{**}p < 0.01$ (unpaired two-tailed Student's t-test).

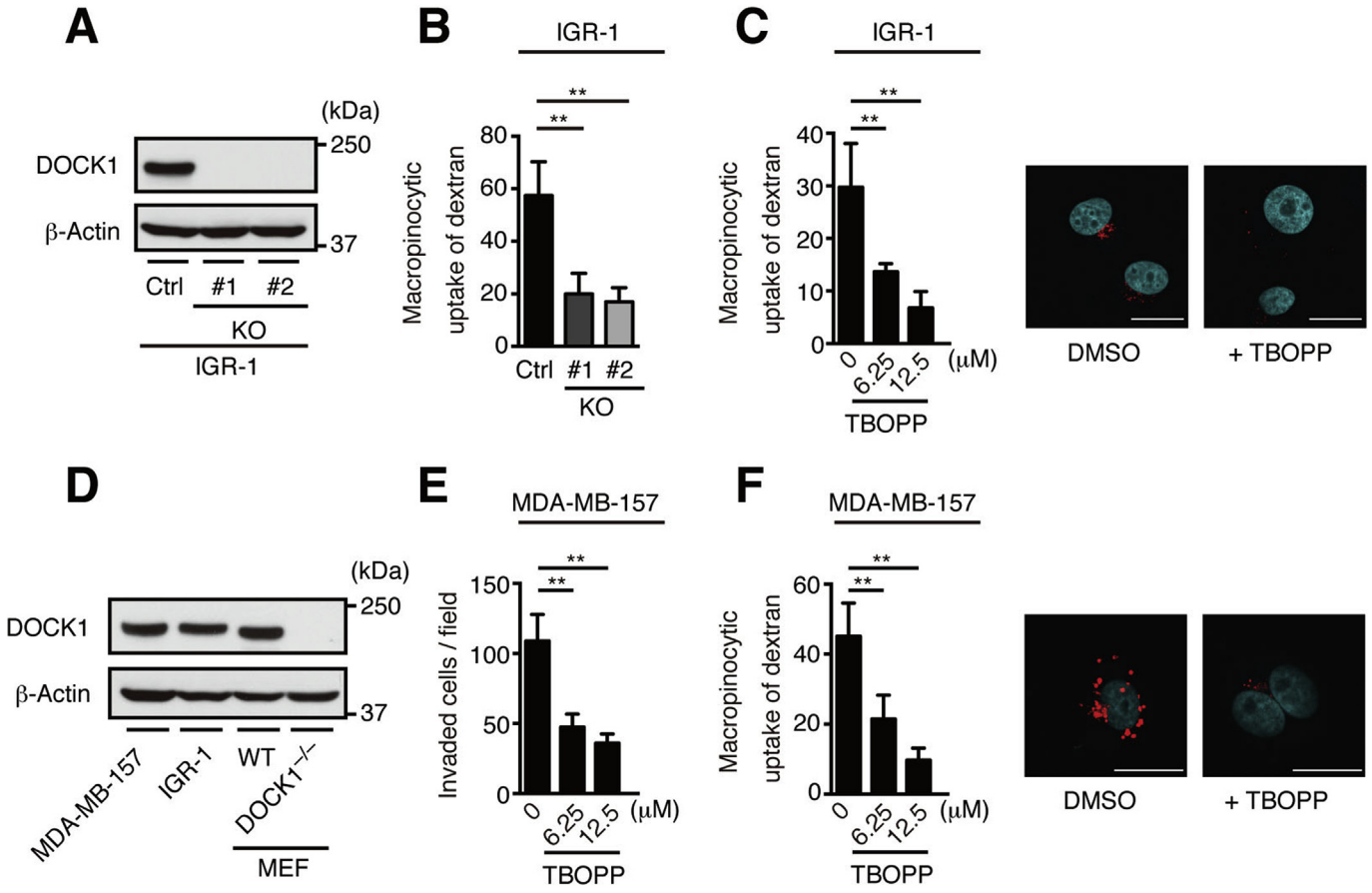

Fig. 4. DOCK1 inhibition suppresses invasion and macropinocytosis in human cancer cells harboring Rac1^{P29S} mutation.
(A) Immunoblots showing the expression of DOCK1 and β-actin in parental IGR-1 (Ctrl) and its DOCK1-KO clones (KO).
(B) Comparison of macropinocytic activity among IGR-1 and its DOCK1-KO clones. Data are means ± SD of five independent experiments. **$p < 0.01$ (unpaired two-tailed Student's t-test).
(C) (Left) Macropinocytic activity of IGR-1 treated with TBOPP (6.25, and 12.5 μM) or DMSO (0, vehicle control) was compared. Data are means ± SD of five independent experiments. **$p < 0.01$ (Mann-Whitney *U* test). (Right) Representative images of IGR-1 treated with DMSO or TBOPP (6.25 μM). Scale bar, 20 μm.
(D) Immunoblots showing the expression of DOCK1 and β-actin in MDA-MB-157, IGR-1, and WT and DOCK1−/− MEFs.
(E) Invasion activity of MDA-MB-157 treated with TBOPP (6.25, and 12.5 μM) or DMSO (0, vehicle control) was compared. Data are means ± SD of five independent experiments. **$p < 0.01$ (unpaired two-tailed Student's t-test).
(F) Effect of TBOPP on the macropinocytic activity of MDA-MB-157. (Left) Macropinocytic uptake of dextran in MDA-MB-157 treated with TBOPP (6.25, and 12.5 μM) or DMSO (0, vehicle control) was compared. Data are means ± SD of five independent experiments. **$p < 0.01$ (Mann-Whitney *U* test). (Right) Representative images of MDA-MB-157 treated with DMSO or TBOPP (6.25 μM). Scale bar, 20 μm.

3.4. DOCK1 inhibition suppresses invasion and macropinocytosis in human cancer cells harboring Rac1^{P29S} mutation

A human skin melanoma cell line IGR-1 and breast cancer cell line MDA-MB-157 have been reported to harbor Rac1^{P29S} mutation [14,25] (Supplemental Fig. 1). Interestingly, we found that both cell lines also express DOCK1 endogenously (Fig. 4A and D). Therefore, we explored the role of DOCK1 in their malignant phenotypes. We first generated DOCK1-deficient (DOCK1-KO) IGR-1 by genome editing (Fig. 4A). Although IGR-1 did not show invasion activity (data not shown), the cells exhibited marked macropinocytic activity (Fig. 4B, Ctrl). In contrast, DOCK1-KO IGR-1 showed significantly decreased macropinocytic activity (Fig. 4B; 67.8% reduction in average compared to that of parental IGR-1). Consistently, the macropinocytic activity of IGR-1 was suppressed by the treatment with TBOPP (Fig. 4C). Similarly, treatment with TBOPP effectively suppressed the invasion and macropinocytosis in MDA-MB-157 (Fig. 4E and F, respectively). Taken together, these results demonstrate that DOCK1 inhibition can suppress the malignant phenotypes of human cancer cells harboring Rac1^{P29S} mutation.

4. Discussion

Rac1^{P29S} has been shown to induce membrane ruffling, migration, anchorage-independent growth, proliferation, and gene expression in various cell types [13–15]. We found that Rac1^{P29S} induces additional cancer-related phenotypes, invasion and macropinocytosis, in mouse embryonic fibroblasts (MEFs). Moreover, we show that DOCK1 acts as a critical Rac GEF for Rac1^{P29S}-induced transformed phenotypes in MEFs and human cancer cells. Thus, for the first time, our study reveals that the cellular functions of Rac1^{P29S} still require the action of an upstream regulator, namely, the GEF DOCK1.

Proline 29 (P29) of Rac1 resides in the conserved Switch I loop, which is important for coordinating nucleotide contacts [14,15]. The increased inherent GDP/GTP exchange activity of Rac1^{P29S} is a result of increased rate of GDP dissociation, presumably due to destabilized GDP-bound state [14,15]. Most common oncogenic mutations of Ras family proteins, such as G12D and Q61L, block the GTPase activity, thereby keeping the mutants in a GTP-bound active state constitutively [26]. On the other hand, Rac1^{P29S} maintains a

normal rate of GTPase activity, and thereby exhibits 'fast-cycling' behavior [14,15]. Thus, the activity of $Rac1^{P29S}$ still could be controlled by the actions of GEFs and GAPs. Indeed, we found that DOCK1 greatly accelerates the GDP/GTP exchange on $Rac1^{P29S}$ (Fig. 1). The DOCK family proteins contain a conserved valine residue that functions as a nucleotide sensor in the α10 helix of the DHR-2 domain [27,28]. The valine contributes to release of GDP from cognate small GTPase via magnesium exclusion, and then to discharge of the activated GTP-bound state via repulsion by GTP-Mg^{2+} [27,28]. This mechanism of GEF reaction is likely to operate synergistically with the increased nucleotide exchange of $Rac1^{P29S}$.

We show that DOCK1 inhibition can suppress invasion and macropinocytosis in WT MEFs expressing $Rac1^{P29S}$ (Figs. 2 and 3) and in human cancer cells harboring $Rac1^{P29S}$ (Fig. 4). The mechanisms by which DOCK1 regulates the transforming activity of $Rac1^{P29S}$ are considered twofold. First, although $Rac1^{P29S}$ possesses increased nucleotide exchange activity, much higher than that of $Rac1^{WT}$, it may not be sufficient to generate a level of GTP-bound active state for transformation within the cells where GAPs counteract by stimulating its GTP hydrolysis for inactivation. In such case, the GEF activity of DOCK1 should drive shifting the balance of nucleotide states of $Rac1^{P29S}$ toward the GTP-bound state. Of note, we previously showed that the Rac GEF activity of DOCK1 is far more potent than that of the Dbl family proteins [23,24]. Secondly, DOCK1 may play an important role in the control of intracellular localization of $Rac1^{P29S}$. DOCK1 associates with two phospholipids, phosphatidylinositol 3,4,5-triphosphate (PIP_3) and phosphatidic acid (PA), through the DHR-1 domain and carboxy-terminal polybasic cluster, respectively [17]. PIP_3 is a product of phosphoinositide 3-kinases, and PA is generated by the action of phospholipase D (PLD), and both are important for the formation of circular dorsal ruffles [8,9,18]. The association with PIP_3 and PA localizes DOCK1 to the membrane structures such as circular dorsal ruffles and invadopodia. In turn, DOCK1 would recruit and activate $Rac1^{P29S}$ for generation of invadopodia and circular dorsal ruffles. Thus, the two unique features of DOCK1, a potent GEF that associates with PIP_3 and PA, would make it a special Rac GEF in the context of cancerous transformation.

More recently, two additional biological functions of $Rac1^{P29S}$ with clinical implications have been reported [25,29]. $Rac1^{P29S}$ confers resistance to RAF inhibitors in melanomas [25]. $Rac1^{P29S}$ also up-regulates PD-L1 expression in melanoma, suggesting a role in the suppression of antitumor immune response [29]. It would be interesting to examine whether these functions of $Rac1^{P29S}$ are also mediated by DOCK1. Given the prevalence of cancers with aberrant expression and activity of Rac1, significant efforts have been undertaken for targeting the small GTPase or its regulatory mechanisms pharmacologically [30]. Our study reveals that targeting an upstream regulator DOCK1 might be an effective approach to control cancer malignancy induced by self-activating Rac1 mutation.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.02.073.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.02.073.

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