**Oncogenes and Tumor Suppressors**

**NEDD9 Regulates 3D Migratory Activity Independent of the Rac1 Morphology Switch in Glioma and Neuroblastoma**

Jessie Zhong¹,², Cuc T. Bach¹, Michael S.Y. Shum¹, and Geraldine M. O’Neill¹,²

**Abstract**

Metastasizing tumor cells must transmigrate the dense extracellular matrix that surrounds most organs. The use of three-dimensional (3D) collagen gels has revealed that many cancer cells can switch between different modes of invasion that are characterized by distinct morphologies (e.g., rounded vs. elongated). The adhesion protein NEDD9 has the potential to regulate the switch between elongated and rounded morphologies; therefore, its role was interrogated in the invasion switch of glioblastoma and neuroblastoma tumors that similarly derive from populations of neural crest cells. Interestingly, siRNA-mediated depletion of NEDD9 failed to induce cell rounding in glioma or neuroblastoma cells, contrasting the effects that have been described in other tumor model systems. Given that Rac1 GTPase has been suggested to mediate the switch between elongated and rounded invasion, the functionality of the Rac1 morphology switch was evaluated in the glioma and neuroblastoma cells. Using both dominant-negative Rac1 and Rac1-specific siRNA, the presence of this morphologic switch was confirmed in the neuroblastoma, but not in the glioma cells. However, in the absence of a morphologic change following NEDD9 depletion, a significant decrease in the cellular migration rate was observed. Thus, the data reveal that NEDD9 can regulate 3D migration speed independent of the Rac1 morphology switch.

**Implications:** NEDD9 targeting is therapeutically viable as it does not stimulate adaptive changes in glioma and neuroblastoma invasion. *Mol Cancer Res; 12(2): 264–73. ©2013 AACR.*

**Introduction**

The adhesion docking protein NEDD9/HEF1/Cas-L is a member of the Cas family of proteins that includes p130Cas/Bcat1, Efs/Sin, and HEPL/CASS4 (1). Increasing studies point to a critical role for NEDD9 as a regulator of migration and invasion in diverse cancer types (2). In addition to this pathologic role, NEDD9 was first identified in a screen for neural precursor cell and developmentally downregulated genes (3) and was subsequently shown to regulate migration in neural precursor/nerve crest cells (4). The neural crest cells are a highly migratory cell type that give rise to a variety of cell lineages that include peripheral neurons, glia, and melanocytes (5). In turn, transformation of neural crest cell populations is thought to give rise to neuroblastoma, glioma brain tumors, and melanoma. Progression to invasive disease remains a significant impediment to successful treatment for each of these tumor types. The neuroblastomas are the most common extracranial tumor of childhood and the majority of patients present with metastatic disease. In contrast to other tumor types, gliomas rarely metastasize to secondary sites but instead the high-grade gliomas (grades 3 and 4) extensively infiltrate the surrounding health brain tissue. Based on recent insights into the role of NEDD9 in the invasive behavior of melanoma (6, 7), we have investigated the role of NEDD9 in glioma and neuroblastoma invasion that exhibits the features of invading neural crest cells.

NEDD9 is expressed during embryonic brain development but is downregulated in the normal adult brain (3) upon lineage commitment of progenitor cells (8). NEDD9 is specifically expressed in nestin-positive neural crest cells that emigrate from the dorsal tube (4). The characteristic invasive morphology of the neural crest cells is suggested to closely resemble that of invasive glioma cells (9, 10). Uniquely among the Cas family proteins, NEDD9 was required for the invasion of U87MG glioblastoma cells through brain homogenates (11). NEDD9 is elevated in high-grade glioma cells and high-level expression in low-grade glioma correlated with significantly poorer prognosis and increased invasion (12). In contrast, the morphologic regulation of invasive neuroblastoma is comparatively less well defined. Notably, NEDD9 was defined as an all transretinoic acid responsive gene upregulated in response to retinoic acid–induced neuritogenesis in neuroblastoma cells (13).

Three-dimensional (3D) collagen gels that mimic the in vivo interstitial environment have been key in demonstrating...
the plasticity of cancer cell invasion mechanisms (7, 14, 15). It has emerged that cancer cells may use differential modes of invasion characterized by distinct cellular morphologies. In particular, NEDD9 promotes elongated/mesenchymal invasion in melanoma cells (7). Elongated invasion is regulated by Rac GTPase activity and Rac GTPase inhibition stimulates the switch to a rounded/amoeboid, Rho kinase–dependent mode of invasion. NEDD9 stimulates Rac GTPase activity via the recruitment of the Rac GTP exchange factor Dedicator of cytokinesis 3 (DOCK3) (7) and concurrently inhibits Rho-associated kinase (ROCK) activation via an Src/αvβ3-dependent mechanism (16). Depletion of NEDD9 from melanoma cells (7) and mouse embryo fibroblasts (17) leads to a switch from an elongated phenotype to a rounded cell phenotype in 3D collagen cultures.

A number of studies have identified a key role for Rac activity in regulating high-grade glioma invasion. Although Rac1 depletion reduced glioma cell line invasion (18), this treatment alone was insufficient to induce a switch to a rounded invasion mode (15). It was only following inhibition of all Rac activity via knockdown of both Rac1 and Rac3 isoforms or expression of dominant-negative Rac (DNRac) that U87MG cells adopted the classic rounded invasion mode (15). Similarly, simultaneous Rac1 and Rac3 depletion, together with Rho kinase inhibition, was required to comprehensively arrest 3D collagen invasion by highly invasive cells isolated from the peritumoral region of a high-grade glioma (19). Although no reports seem to exist between NEDD9 expression and poor survival rates for glioma (20), NEDD9 depletion failed to induce a morphologic switch in either high-grade glioma or neuroblastoma 3D collagen cultures. Notably, although the neuroblastoma cells exhibit a significant association with poor survival rates for both tumor types. Importantly, in contrast to effects seen in melanoma, NEDD9 depletion fails to induce a morphologic switch in either high-grade glioma or neuroblastoma cells. Notably, although the neuroblastoma cells exhibit a robust morphologic switch in response to Rac inhibition, this effect is much reduced in the high-grade glioma cells. Instead, we find that NEDD9 depletion significantly reduces migration speed of cells as they negotiate the 3D collagen environment. Collectively, our data reveal that NEDD9 plays a significant role in regulating cell speed in 3D environments, independently of the Rac-mediated morphologic switch.

Materials and Methods

Cell culture and antibodies

Cultured glioblastoma (A172, CCF-STTG1, DBTRG, MO59J, MO59K, and T98G) cell lines were kindly provided by Dr. Kerrie McDonald (Lowy Cancer Centre, UNSW, Australia). Correct identity of A172 and DBTRG lines was independently verified by CellBank Australia. The neuroblastoma cell lines (NB69, SH-EP, SH-SY5Y, SK-N-AS, SK-N-FI, and SK-N-SH) were kindly provided by Dr. Loretta Lau (Kids Research Institute, Sydney, Australia). Cell lines were maintained in Dulbecco’s modified eagle’s medium (DMEM) supplemented with 10% FBS. Growth of cells in 3D collagen gels, media for live imaging, and invasion assays through 3D collagen gels was based on protocols previously reported (21) and as we have previously used (22). The following antibodies were used: anti-NEDD9 (ImmuQuest; clone 2G9 and Cell Signaling Technology, clone 29G), anti-Rac-α, anti-β, and anti-α-tubulin (Sigma-Aldrich), and horseradish peroxidase–conjugated anti-mouse and anti-rabbit (Amersham and Biorad) and anti-rat Alexa-Fluor 647 conjugate (Molecular Probes; Invitrogen).

Protein extraction, siRNA, and immunoblotting

Conditions of protein extraction and immunoblotting were carried out as previously described (23). Protein concentrations were determined using the BCA Protein Assay Kit (Pierce Biotechnology) and protein concentrations equalized before loading on gels. Control siRNA and custom-designed NEDD9 siRNAa [targeting the sequence GGACACCAUCGUACAAA, as previously described (17)] and siR Nab [GAGACACAUUCCAAGUU, as previously described (17)] were purchased from Dharmacon (Thermo Scientific). Of note, 10 nmol/L of siRNA was transiently transfected twice at 24-hour intervals, using Lipofectamine2000 (Life Technologies) as per the manufacturer’s instructions. Inhibition of Rac GTPase activity was achieved by transfecting cells with plasmid vector encoding DNARac as previously described (24). Custom-designed Rac siRNAs were purchased from Invitrogen comprising sequences targeting human Rac1 (5′-GAGGCCUCAAGACAGUGUUGAAGCA-3′) and Rac3 (5′-CCUCGGCGGACGACAGGACAGACU-3′); control sequences for Rac knockdown experiments were Qiagen Allstar Non-targeting Control siRNA (Qiagen). Rac siRNAs were used at a final concentration of 100 nmol/L when used individually and 50 nmol/L each when used in combination. Successful knockdown was independently confirmed for all experiments.

Live cell imaging, migration, and rounding analysis

Time-lapse images were captured using an ORCA ERG cooled CCD camera (Hamamatsu; SDR Clinical Technology) and Olympus IX81 inverted microscope equipped with an environmental chamber heated to 37°C. Transmitted light images were captured at indicated time intervals. Cells undergoing division or apoptosis were excluded from analyses and random migration analyses were performed on sparsely plated cultures. After image capture, nuclear translocation was tracked in time-lapse stacks using Metamorph V6.3 software (Molecular Devices). Calculation of mean squared displacement (MSD) was performed as previously
described (25). We note that only elongated cells were tracked in the analysis of 3D migration. Glioblastoma cell morphologies in 3D collagen gels were manually scored as either elongated, displaying extending protrusions and an elongated cell body, or rounded, with a rounded cell body and the absence of protrusions. Cells were counterstained with propidium iodide (Sigma-Aldrich) and apoptotic cells excluded from morphologic analyses. Reduced cell branching exhibited by the SH-EP cells facilitated semiautomated analysis of cell shapes. In this case, z-stacks of fluorescently tagged phalloidin-stained cells were captured using a Leica SP5 confocal scanning laser microscope (Leica Microsystems) with 10× air objective. Maximum projections of z-stacks were filtered using the Metamorph V7.7 software by Fast Fourier Transformation followed by low-pass basic filter, auto-thresholding for light objects, and drawing regions around objects. Any joined cells were manually separated, and the cell shape factor \(4\pi A/P^2; A = \text{area, } P = \text{perimeter}\) was then measured using integrated morphology analysis. Data were filtered to exclude anything smaller than 15 pixels in area. Cells with a shape factor of \(>0.75\) were scored as rounded.

Image preparation
Final micrograph images and gray level adjustments were prepared in Adobe Photoshop.

Results
NEDD9 expression in glioma and neuroblastoma
We first examined the relationship between NEDD9 expression and patient survival in both glioma and neuroblastoma. This was achieved using publicly available data from the REpository for Molecular BRAin Neoplasia DaTa (REMBRANDT) homepage (http://rembrandt.nci.nih.gov) and the Oncogenomics website (http://home.ccr.cancer.gov/oncology/oncogenomics/) for neuroblastoma. In agreement with previously published results (12), high-level NEDD9 expression was significantly associated with poorer survival rates in a cohort of glioma patients (Fig. 1A). Moreover, comparison of the percentage of low-grade gliomas (grade 2/3) and high-grade gliomas (grade 4, glioblastoma) between cases with intermediate versus high-level NEDD9 expression revealed significantly more high-grade glioma cases in the high NEDD9 dataset (Fig. 1B). Notably, we found a significant association between high NEDD9 expression and patient survival in both glioma and neuroblastoma.
expression and reduced patient survival in the subset of neuroblastomas that lack N-myc amplification (n = 101; Fig. 1C). Based on these observations, NEDD9 expression was then profiled in human glioblastoma cell lines, and neuroblastoma lines that lack N-myc amplification. The highest level of NEDD9 expression (seen as a doublet on Western blot; ref. 26) was detected in the A172, DBTRG, and MO59J glioblastoma cells and in the SH-EP and SK-N-SH neuroblastoma cells (Fig. 1D). The SK-N-SH cells are a mixed culture from which both the SH-EP and SH-SY-5Y cell lines were derived (27). It is therefore interesting that although the SH-EP and SK-N-SH lines are positive for NEDD9, the SH-SY-5Y cells exhibit little detectable NEDD9, suggesting that SK-N-SH cells may be a mix of high and low NEDD9-expressing cells.

**NEDD9 depletion does not induce rounding**

It has previously been established that NEDD9 regulates the morphologic switch from elongated to rounded 3D invasion in melanoma cells (7). Thus, we questioned the effect of NEDD9 depletion on glioblastoma cell morphology, focusing on the A172 and DBTRG cells due to their high endogenous levels of NEDD9 expression. Under 3D culture conditions, approximately 60% of the A172 cells and approximately 40% of DBTRG cells have an elongated morphology (Fig. 2B and C). Inspection of cells following NEDD9 depletion suggested little evidence of a switch to a rounded morphology in either cell line, despite robust NEDD9 depletion (Fig. 2A and B). Rather, quantification indicated that NEDD9 depletion caused a small decrease in the percentage of round A172 and DBTRG cells (Fig. 2C). Thus, NEDD9 depletion does not seem to induce rounding in either A172 or DBTRG glioblastoma cells.

We next questioned the effect of NEDD9 depletion on neuroblastoma cell morphology, using the SH-EP line that has high endogenous NEDD9 expression (Fig. 1C). In 3D collagen gels, the SH-EP cells have a mixed morphology with approximately 50% of cells exhibiting an

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**Figure 2.** NEDD9 depletion does not cause cell rounding. A, Western blot analysis with anti-NEDD9 antibodies showing depletion of NEDD9 protein in A172 and DBTRG cells following NEDD9-targeted siRNA. Blots were probed with anti-HSP70 antibodies to demonstrate equal loading. B, A172 and DBTRG cell morphology in 3D collagen gels showing examples of elongated cells (arrow heads) and rounded cells (arrows). C, histogram shows the percentage of rounded cells under the indicated conditions. Cells were counterstained with propidium iodide and apoptotic cells excluded from morphologic analyses. Data averaged from >20 fields of view with 4× objective. **P < 0.01, Student t test.** D, morphology of SH-EP cells in 3D collagen gels under the indicated treatment conditions. Micrographs show maximum projections of confocal slices of the entire gel containing cells fixed and stained with fluorescently tagged phalloidin. E, Western blot analysis with anti-NEDD9 antibodies showing depletion of NEDD9 protein in SH-EP cells following NEDD9 targeting with two independent siRNAs (a and b). Blots were probed with anti-HSP70 antibodies to demonstrate equal loading. F, histogram shows the average numbers of rounded cells under each condition. Data represent the average from 3 fields of view, >250 cells per field. *, P < 0.05, Student t test.
Differential response to Rac1 inhibition

We considered that the lack of effect of NEDD9 depletion on the cell morphologies may reflect absence of the morphologic switch (elongated/mesenchymal to rounded/amoeboïd) in these cells. Rac GTPase is a major regulator of the switch between elongated and rounded invasion (7, 15) and NEDD9 is an upstream regulator of this pathway in melanoma cells (7). Thus, we tested whether inhibition of Rac GTPase activity via the expression of dominant-negative Rac (GFP.DNRac) induced morphologic transition in either the A172 or SH-EP cells. DNRac resulted in only a modest increase in the numbers of rounded A172 glioblastoma cells (Fig. 3A). By contrast, approximately 80% of SH-EP cells expressing GFP.DNRac exhibited a rounded phenotype (Fig. 3A). This is highlighted in 10 random fields of view for GFP versus GFP.DNRac-transfected SH-EP cells in which the GFP-positive control cells display a mix of elongated and rounded morphologies, whereas all visible GFP.DNRac-positive cells are rounded (Fig. 3B). These data suggest that although the Rac-dependent morphology switch is strongly present in the SH-EP cells, the loss of Rac activity seems to have less effect in the A172 cells.

Previous studies targeting Rac isoforms revealed that switching U87MG glioblastoma cells from an elongated to a rounded morphology requires the depletion of both Rac1 and Rac3 isoforms (15). This suggested the possibility that the failure to induce rounding in A172 cells following NEDD9 depletion may be due to NEDD9 regulating a specific isoform of Rac and that the presence of other Rac isoforms may therefore compensate. Rac 3 is highly expressed in the brain (28) and both Rac1 and Rac3 are suggested to play a key role in glioblastoma invasion (29). Thus, we questioned whether A172 rounding may require the inhibition of both Rac1 and Rac3 isoforms. Depletion was achieved using siRNA specific for each Rac isoform as previously described (15). Due to the absence of commercially available isoform-specific antibodies, confirmation of knockdown was determined by comparing the levels of total Rac protein (Fig. 4A). Notably, only siRNA targeting Rac1, not Rac3, significantly decreased levels of total Rac protein (Fig. 4B). Because it has previously been suggested that Rac3 depletion may lead to a corresponding increase in Rac1 protein (15), we proceeded with the double knockdown of Rac1 and Rac3 and examined the morphology of cells resulting from all three siRNA conditions. This analysis revealed that combined depletion of Rac1 and Rac3 had no effect on cellular morphologies in 3D collagen gels (Fig. 4C and D). Similarly, individual targeting of the Rac isoforms had no effect on A172 morphology. It is interesting that Rac knockdown was not able to recapitulate the small increase in rounding that was seen in cells expressing DNRac (Fig. 3), despite a significant reduction in total Rac protein levels (Fig. 4A). Together, these data emphasize that the A172 cells have a limited response to the Rac-dependent morphology switch.

Given that the Rac morphology switch seems to be robustly functioning in the SH-EP cells, we next questioned the isoform-specific role of Rac in SH-EP 3D morphology. Although Rac1 depletion significantly reduced total Rac

Figure 3. DNRac induces cell rounding. A, histogram showing the percentages of cells (A172 and SH-EP, as indicated) transfected with GFP control vector or GFP fused to DNRac displaying a rounded phenotype. Data represent the average from triplicate repeats, >60 cells scored per condition, per experiment. *, P < 0.05; **, P < 0.01; Student t test. B, bright field images of SH-EP cells in collagen overlayed with GFP fluorescence (10 examples shown per condition). Top, cells transfected with GFP empty vector; bottom, with GFP.DNRac. Scale bar, 50 μm.
protein levels, Rac3 depletion had no effect (Fig. 5A and B), suggesting either that Rac3 may not be expressed in the SH-EP cells, or there is a compensating increase in Rac1, as suggested earlier. Critically, Rac1 depletion significantly increased the numbers of rounded SH-EP cells (Fig. 5C and D), mimicking the effect of DNRac. In summary, these data suggest that the morphologic switch in SH-EP cells is Rac1 dependent.

**NEDD9 depletion slows 3D migration.**

Our investigations have revealed distinct differences in the morphologic control of A172 glioblastoma and SH-EP cells in 3D collagen gels, despite both lines expressing high levels of endogenous NEDD9 protein. Moreover, the data suggest that NEDD9 does not regulate a Rac1-dependent molecular switch in these cell lines. This then raises the question of whether NEDD9 plays any role in the 3D migration of these cells. To examine this question, we measured 3D migration speed by analysis of time-lapse images of the cells as they migrate through the 3D collagen gels. This revealed that the A172 cells move more slowly through the gels following NEDD9 depletion. Reduced NEDD9 levels caused a shift in the MSD and a significant decrease in average cell speed (Fig. 6A and B). Similarly, the DBTRG cells (that also do not undergo rounding following NEDD9 depletion, as demonstrated in Fig. 2) slow down following NEDD9 depletion (Fig. 6A and B). Finally, we also observed that the SH-EP cells slow down in response to treatment with either siRNAa or siRNAb targeting NEDD9. When tracking the SH-EP cells following NEDD9 depletion, there seemed to be two populations of cells. One fast moving group seemed to be relatively unaffected by NEDD9 depletion. However, the slower moving group was more affected. Comparison of the frequency distribution of speeds between the different conditions revealed that the greatest changes occurred at speeds ≤0.4 μm/min (Fig. 6C). Accordingly, the average speed was significantly lower in the <0.4 μm/min populations treated with either NEDD9-targeting siRNA (Fig. 6D). Notably, following NEDD9 knockdown, the cells continued to display rounded cell bodies with a leading membrane protrusion, with occasional pausing as the cells retracted the protrusion and adopted a rounded shape, but had slower progress through the gel (Fig. 6E). Together, these data reveal that NEDD9 depletion reduces 3D migration speed, without stimulating transition to rounded invasion.

**Discussion**

Based on insights from developmental biology, in the present study, we have investigated the role for NEDD9 in the migration of tumors that derive from neural crest cell populations. We used 3D collagen gel models to investigate invasion plasticity in response to altered NEDD9 expression (7, 21). Importantly, although we show cell-type differences in regard to invasion plasticity control by...
Rac GTPase activity, the cell lines investigated in the present study are united by their lack of morphologic switch following NEDD9 depletion. Thus, our data suggest that NEDD9 may not universally regulate the transition between morphologically distinct invasion phenotypes. Instead, we find that NEDD9 is an important determinant of the speed with which cells negotiate the 3D extracellular environment.

Before initiating investigations of NEDD9 and migration in glioma and neuroblastoma cell lines, we first determined whether there is any clinical association between NEDD9 and survival in these tumor types. Related to this, a recent study showed that higher-level NEDD9 in lower-grade glioma tumors is associated with reduced progression-free survival (12). In agreement with this, analysis of REMBRANDT data presented here demonstrated an association between NEDD9 expression and survival when all glioma tumors were grouped together. When NEDD9 levels were examined specifically in the high-grade gliomas (3 and 4), there was no significant association between expression and survival (data not shown). Importantly, recent advances in tumor profiling have revealed that high-grade gliomas can be further stratified into risk groups based on gene expression profiles. For example, one such classification scheme defines cells as proneural, proliferative, and mesenchymal, with corresponding increase in aggressiveness (30). Potentially, NEDD9 expression may prove to be more prominent in one of these subclasses, particularly given recent reports suggesting a correlation between NEDD9 and nestin expression in glioma (12). We observed a striking inverse relationship between NEDD9 expression and patient survival in the subset of neuroblastomas that lack N-myc amplification.

Critically, approximately 65% of patients with metastatic disease have no N-myc amplification and variable clinical behaviors of these tumors complicate treatment decisions; thus, there is an important need for molecular markers that can stratify these tumors (31). Together, these analyses expand the range of tumor types in which high-level NEDD9 expression has been correlated with reduced patient survival rates including melanoma (6), lung adenocarcinoma (32–34), and head and neck cancer (35).

The biophysical constraints supplied by the 3D collagen gel model allow interrogation of invasion plasticity as cells adopt discrete morphologies (rounded vs. elongated) reflective of the underlying invasion machinery. Using this approach, we have shown that NEDD9 depletion failed to induce a switch in cellular morphologies in any of the cell lines under investigation. Notably, siRNA targeting NEDD9 is identical to the sequence previously successfully used to induce a switch to rounded invasion in melanoma cells (equivalent to NEDD9 OT-4–targeting sequence; ref. 7). Thus, it seems that there may be cell-type–specific...
differences in the effects of NEDD9 on invasion plasticity. Importantly, we confirmed that the SH-EP cells exhibit a robust switch to rounded morphology following Rac1 depletion. Thus, these cells are clearly competent to undergo morphologic transition. By contrast, the A172 glioblastoma cells exhibited limited morphologic transition following Rac inhibition. The small morphologic change in A172 seen following DNRac inhibition is likely due to the well-described nonspecific inhibition of other Rho family GTPases following high-level expression of this construct (36).

In contrast to our data, other high-grade glioma cell lines such as the U87MG cells undergo morphologic transition to rounded invasion following Rac1 inhibition (15). Both the A172 and DBTRG cell lines are positive for nestin and delta-like 3 (DLL3) markers that are associated with the proneural phenotype. Potentially, the ability of cells to switch between invasion modes (and use different routes of invasion throughout the brain) may also prove to track with subcategories of high-grade glioma. In contrast to the A172 cells, the SH-EP cells undergo morphologic transition following Rac1 inhibition, but this is not phenocopied by NEDD9 inhibition. Similarly, we have previously shown that adoption of an arborized phenotype with neuritic membrane extensions is induced by combined NEDD9 and Rho kinase inhibition and cannot be blocked by Rac inhibition (24). Thus, at least in these two examples it seems that NEDD9 may not be regulating cellular morphologies via activation of Rac1 GTPase.

The striking finding from our study is that in each case NEDD9 depletion significantly reduced the speed of 3D cell migration. Importantly, this suggests a similar mechanism of NEDD9 action in these tumor types from diverse backgrounds. The 3D collagen matrix requires cells to either deform themselves to squeeze through pores in the matrix (rounded invasion) or alternatively deform the matrix via exerting force through adhesion to the matrix and secreting enzymes to degrade the matrix (elongated invasion; ref. 37). Significantly, NEDD9 depletion reduced speed, without altering elongated morphologies.

Similarly, let-7i microRNA-mediated inhibition of the Twist/NEDD9/Rac1 signaling pathway in head and neck cancer cells reduced the speed of elongated cell migration (35). We have previously shown that mouse embryo fibroblasts lacking NEDD9 expression exhibited reduced adhesive force to the extracellular matrix (17). Thus, it is possible that the reduced migration speed exhibited following NEDD9 depletion may be due to decreased adhesive force to the surrounding matrix. Alternatively, given that NEDD9 has previously been shown to modulate matrix metalloproteinase expression (38), NEDD9 depletion may reduce matrix reorganization. Collectively, the common effect of NEDD9 on 3D cell speed suggests differences in the effects of NEDD9 on invasion plasticity.

Figure 6. NEDD9 depletion reduces 3D migration speed. A, MSD calculated from trajectories of control (black squares) and NEDD9 siRNA-treated cells (white squares) in the indicated cell lines, migrating in 3D collagen gels. Data represent the average of 3 (A172) and 5 (DBTRG) independent experiments (approximately 50 cells tracked per experiment). B, 3D cell speed is significantly reduced following NEDD9 siRNA. **, P < 0.001; ***, P < 0.0001, Student t test. C, frequency distribution of SH-EP cell speeds following NEDD9 depletion as indicated. D, comparison of the average SH-EP cell migration speeds <0.4 μm/min. **, P < 0.001; ***, P < 0.0001, Student t test. E, representative images from time-lapse series of SH-EP neuroblastoma cells in 3D collagen gels, showing characteristic morphologies under control conditions and following NEDD9 siRNA. Frames shown are from 0.5 hourly time intervals as indicated. Arrow heads point to the cell of interest. Scale bar, 20 μm.
that targeting NEDD9 may be a useful therapeutic target in subsets of glioma and neuroblastoma tumor cells with high NEDD9 expression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: C.T. Bach, G.M. O’Neill
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Zhong, C.T. Bach, M.S.Y. Shum
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Zhong, C.T. Bach, M.S.Y. Shum, G.M. O’Neill

References

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