Data Article

Data on the negative regulation of invadopodia activity by MLCK

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1. Data

Actomyosin-generated contractile forces promote ECM degradation by proteolytic subcellular protrusions called invadopodia [1,2]. Cellular contractility can be regulated by several kinases including MLCK; however, we and others have found that MLCK inhibition does not always affect force generation [1,3,4]. Despite this finding in our laboratory using SCC-61 cells, we show here that KD of MLCK increased ECM degradation by SCC-61 cells (Fig. 1A and B) as well as the number of invadopodia formed. These data are related to the research article entitled “Matrix rigidity differentially regulates invadopodia activity through ROCK1 and ROCK2” Jerrell and Parekh, 2016.

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actively degrading ECM (i.e., colocalized with ECM degradation; Fig. 1A and C) and the total number of invadopodia (i.e., actively degrading and non-degrading or not colocalized with ECM degradation; Fig. 1A and D) when compared to NTC in the rigid PAA invadopodia assay which approximates high grade tumor rigidity (raw data presented in Table 1). Western blot data confirming MLCK KD in SCC-61 cells used in these experiments was previously reported and technically described [1].

2. Experimental design, materials, and methods

2.1. Cell culture and MLCK inhibition

SCC-61 cells were cultured as previously described as well as KD of MLCK with siGENOME SMARTpool siRNA (ThermoScientific) or the NTC following the manufacturer’s protocol to maximize inhibition while minimizing off-target effects [1].

2.2. Rigid PAA invadopodia assay

Rigid PAAs were synthesized and cast on activated coverslips of 35 mm MatTek dishes as previously described [1]. Briefly, these substrates were composed of a 12%/0.6% ratio of acrylamide/BIS-acrylamide, 0.1% N-hydroxysuccinimide ester, and 230 μg/ml of fibronectin yielding an elastic modulus of 22,692 Pa which mimics tumor rigidity and maximizes invadopodia activity. To detect and evaluate ECM degradation, the rigid PAAs were overlaid with 1% gelatin (crosslinked with glutaraldehyde) and FITC-labeled fibronectin.

2.3. Immunofluorescence

Cells were incubated overnight in invadopodia medium and immunostained in the rigid invadopodia assays as previously described [1]. Briefly, the invadopodia markers actin and cortactin were
identified with Alexa Fluor 546 phalloidin (Life Technologies) and a mouse monoclonal antibody (EMD Millipore), respectively. Fluorescent images were captured on a Nikon Eclipse Ti-E inverted microscope with a 40× Plan Fluor oil immersion lens. Metamorph software (Molecular Devices) was used for image analyses which included thresholding for ECM degradation and manual quantitation of invadopodia.

2.4. Statistics

Statistical analyses were performed on pooled data using SPSS Statistics (IBM) as previously described [1]. Briefly, data did not pass the normality test and were therefore analyzed with a Mann-Whitney test for comparisons between datasets.

Fig. 1. MLCK negatively regulates invadopodia activity. (A) Representative wide-field fluorescence images of NTC and MLCK KD SCC-61 cells in the rigid PAA invadopodia assay in which invadopodia were identified by the colocalization (pink) of actin (red) and cortactin (blue). Actively degrading (active; yellow circles) invadopodia were identified based on the colocalization of these markers with ECM degradation (i.e., black areas lacking FITC signal). Total invadopodia included the active and non-degrading (white circles) invadopodia. Quantitation of the (B) degradation area per cell, (C) active invadopodia per cell, and (D) total invadopodia per cell for NTC versus MLCK KD. Data are presented as box and whisker plots with the black lines indicating the medians, the whiskers representing the 10th and 90th percentiles, and * indicating p < 0.05 for n = 86–97 cells for each condition from 3 independent experiments. Scale bar represents 20 μm.
Table 1

Raw data from the immunofluorescence image analyses of NTC and MLCK KD SCC-61 cells in the rigid PAA invadopodia assay.

| Experiment | Condition | Replicate | Degradation (µm²) | Degrading Invadopodia | Total Invadopodia |
|------------|-----------|-----------|-------------------|-----------------------|-------------------|
| 1          | NTC       | 1         | 12.7,151,411      | 7                     | 7                 |
| 1          | NTC       | 1         | 6.822,162,246     | 9                     | 9                 |
| 1          | NTC       | 1         | 8.460,459,273     | 4                     | 4                 |
| 1          | NTC       | 1         | 0                 | 0                     | 0                 |
| 1          | NTC       | 1         | 0                 | 0                     | 1                 |
| 1          | NTC       | 1         | 59.14,985,833     | 9                     | 10                |
| 1          | NTC       | 1         | 62.18,193,044     | 14                    | 16                |
| 1          | NTC       | 1         | 0                 | 0                     | 1                 |
| 1          | NTC       | 1         | 0                 | 0                     | 0                 |
| 1          | NTC       | 1         | 0                 | 0                     | 1                 |
| 1          | NTC       | 1         | 0                 | 0                     | 0                 |
| 1          | NTC       | 1         | 4.010,159,887     | 1                     | 1                 |
| 1          | NTC       | 2         | 0                 | 0                     | 4                 |
| 1          | NTC       | 2         | 0                 | 0                     | 1                 |
| 1          | NTC       | 2         | 25.8,948,739      | 23                    | 23                |
| 1          | NTC       | 2         | 12.006,027,47     | 5                     | 6                 |
| 1          | NTC       | 2         | 0                 | 0                     | 2                 |
| 1          | NTC       | 2         | 0                 | 0                     | 3                 |
| 1          | NTC       | 2         | 1.051,444,361     | 2                     | 2                 |
| 1          | NTC       | 2         | 17.01,872,732     | 3                     | 3                 |
| 1          | NTC       | 2         | 9.316,286,078     | 7                     | 7                 |
| 1          | NTC       | 2         | 0.537,948         | 0                     | 0                 |
| 1          | NTC       | 2         | 0                 | 0                     | 2                 |
| 1          | NTC       | 2         | 22.3,982,101      | 10                    | 10                |
| 1          | NTC       | 2         | 29.02,475,479     | 4                     | 4                 |
| 1          | NTC       | 2         | 18.70,592,874     | 14                    | 14                |
| 1          | NTC       | 2         | 8.680,529,023     | 1                     | 4                 |
| 1          | NTC       | 2         | 0                 | 0                     | 0                 |
| 1          | NTC       | 2         | 19.21,942,482     | 9                     | 12                |
| 1          | NTC       | 2         | 4.156,873,053     | 0                     | 7                 |
| 1          | NTC       | 2         | 0                 | 0                     | 6                 |
| 1          | NTC       | 2         | 0                 | 0                     | 7                 |
| 1          | NTC       | 2         | 0                 | 0                     | 1                 |
| 1          | NTC       | 2         | 34.6,976,639      | 10                    | 14                |
| 1          | NTC       | 1         | 7.091,136,385     | 0                     | 10                |
| 1          | NTC       | 1         | 79.39,627,532     | 28                    | 38                |
| 1          | NTC       | 2         | 7.94,696,319      | 2                     | 4                 |
| 1          | NTC       | 1         | 0                 | 0                     | 7                 |
| 1          | NTC       | 1         | 45.18,765,531     | 23                    | 26                |
| 1          | NTC       | 1         | 3.521,115,998     | 1                     | 4                 |
| 1          | NTC       | 1         | 59.17,431,052     | 25                    | 25                |
| 1          | NTC       | 1         | 12.61,733,233     | 3                     | 8                 |
| 1          | NTC       | 1         | 29.416            | 8                     | 17                |
| 1          | NTC       | 1         | 4.08,351,647      | 7                     | 8                 |
| 1          | NTC       | 1         | 1.66,275          | 1                     | 7                 |
| 1          | NTC       | 1         | 207.6,235,829     | 32                    | 42                |
| 1          | NTC       | 1         | 31.81,230,496     | 34                    | 34                |
| 1          | NTC       | 1         | 27.70,433,418     | 8                     | 10                |
| 1          | NTC       | 1         | 0                 | 0                     | 14                |
| 1          | NTC       | 1         | 55.60,429,014     | 43                    | 43                |
| 1          | NTC       | 1         | 12.22,609,722     | 16                    | 22                |
| 1          | NTC       | 1         | 0                 | 0                     | 8                 |
| 2          | NTC       | 2         | 10.88,122,652     | 8                     | 8                 |
| 2          | NTC       | 2         | 45.87,231,675     | 8                     | 22                |
| 2          | NTC       | 2         | 31.83,675,715     | 8                     | 14                |
| 2          | NTC       | 2         | 120.6,960,317     | 32                    | 35                |
| 2          | NTC       | 2         | 3.741,185,748     | 5                     | 7                 |
| 2          | NTC       | 2         | 5.159,413,025     | 0                     | 12                |
| 2          | NTC       | 2         | 10.8,079          | 5                     | 7                 |
| Experiment | Condition | Replicate | Degradation ($\mu m^2$) | Degrading Invadopodia | Total Invadopodia |
|------------|-----------|-----------|-------------------------|-----------------------|------------------|
| 2          | NTC       | 2         | 0                       | 0                     | 9                |
| 2          | NTC       | 2         | 16.82,310,977            | 6                     | 7                |
| 2          | NTC       | 2         | 21.24,895,696            | 7                     | 8                |
| 2          | NTC       | 2         | 65.8,742,118             | 4                     | 5                |
| 2          | NTC       | 2         | 13.27,754,158            | 0                     | 0                |
| 2          | NTC       | 2         | 13.54,651,572            | 0                     | 8                |
| 2          | NTC       | 2         | 15.23,371,713            | 8                     | 14               |
| 2          | NTC       | 2         | 75.2,394                 | 17                    | 18               |
| 2          | NTC       | 2         | 21.9,916,621             | 12                    | 18               |
| 3          | NTC       | 1         | 15.25,816,933            | 8                     | 12               |
| 3          | NTC       | 1         | 2.860,906,749            | 1                     | 4                |
| 3          | NTC       | 1         | 0                       | 0                     | 4                |
| 3          | NTC       | 1         | 0                       | 0                     | 5                |
| 3          | NTC       | 1         | 0                       | 0                     | 0                |
| 3          | NTC       | 1         | 0                       | 0                     | 0                |
| 3          | NTC       | 1         | 12.51,952,355            | 10                    | 11               |
| 3          | NTC       | 1         | 0                       | 0                     | 0                |
| 3          | NTC       | 1         | 0                       | 0                     | 2                |
| 3          | NTC       | 1         | 0                       | 0                     | 2                |
| 3          | NTC       | 1         | 8.436,007,079            | 1                     | 5                |
| 3          | NTC       | 1         | 0                       | 0                     | 1                |
| 1          | MLCK KD   | 1         | 24.40,329,004            | 16                    | 15               |
| 1          | MLCK KD   | 1         | 20.07,525,163            | 9                     | 7                |
| 1          | MLCK KD   | 1         | 0                       | 2                     | 0                |
| 1          | MLCK KD   | 1         | 6.308,666,163            | 5                     | 3                |
| 1          | MLCK KD   | 1         | 28.29,118,896            | 36                    | 26               |
| 1          | MLCK KD   | 1         | 7.65,354                 | 3                     | 3                |
| 1          | MLCK KD   | 1         | 0                       | 5                     | 0                |
| 1          | MLCK KD   | 1         | 21.00443,502             | 5                     | 4                |
| 1          | MLCK KD   | 1         | 3.570,020,387            | 0                     | 0                |
| 1          | MLCK KD   | 1         | 62.01,076,508            | 19                    | 19               |
| 1          | MLCK KD   | 1         | 0                       | 9                     | 0                |
| 1          | MLCK KD   | 1         | 0                       | 3                     | 0                |
| 1          | MLCK KD   | 1         | 24.94,123,832            | 14                    | 13               |
| 1          | MLCK KD   | 1         | 16.9,698                 | 3                     | 3                |
| 1          | MLCK KD   | 1         | 9.31,629                 | 7                     | 7                |
| 1          | MLCK KD   | 1         | 0                       | 2                     | 0                |
| 1          | MLCK KD   | 1         | 0                       | 1                     | 0                |
| 1          | MLCK KD   | 1         | 10.61,225,238            | 15                    | 12               |
| 1          | MLCK KD   | 1         | 111.6,487,198            | 16                    | 16               |
| 1          | MLCK KD   | 1         | 47.8,529,445             | 17                    | 12               |
| 1          | MLCK KD   | 1         | 86.78,083,804            | 9                     | 9                |
| 1          | MLCK KD   | 2         | 4.52,365,597             | 12                    | 5                |
| 1          | MLCK KD   | 2         | 50.90,946,881            | 30                    | 30               |
| 1          | MLCK KD   | 2         | 46.19,019,528            | 10                    | 10               |
| 1          | MLCK KD   | 2         | 25.1,857,568             | 9                     | 8                |
| 1          | MLCK KD   | 2         | 24.01,204,611            | 6                     | 6                |
| 1          | MLCK KD   | 2         | 54.4,305,848             | 22                    | 18               |
| 1          | MLCK KD   | 2         | 85.43,596,734            | 10                    | 6                |
| 1          | MLCK KD   | 2         | 57.60,937,008            | 22                    | 22               |
| 1          | MLCK KD   | 2         | 19.21,942,482            | 5                     | 5                |
| 1          | MLCK KD   | 2         | 13.74,213,327            | 10                    | 10               |
| 1          | MLCK KD   | 2         | 8.020,319,774            | 7                     | 6                |

(continued on next page)
| Experiment | Condition | Replicate | Degradation (μm²) | Degrading Invadopodia | Total Invadopodia |
|------------|-----------|-----------|-------------------|-----------------------|------------------|
| 1          | MLCK KD   | 2         | 2.640,836,999     | 9                     | 0                |
| 1          | MLCK KD   | 2         | 10.07,430,411     | 4                     | 4                |
| 1          | MLCK KD   | 2         | 11.15,020,066     | 6                     | 5                |
| 1          | MLCK KD   | 2         | 33.96,409,807     | 10                    | 6                |
| 1          | MLCK KD   | 2         | 11.90,822,077     | 8                     | 7                |
| 2          | MLCK KD   | 1         | 161.3,111,267     | 18                    | 17               |
| 2          | MLCK KD   | 1         | 401.0159,887      | 47                    | 43               |
| 2          | MLCK KD   | 1         | 72.64,746,966     | 35                    | 23               |
| 2          | MLCK KD   | 1         | 41.86,215,687     | 12                    | 7                |
| 2          | MLCK KD   | 1         | 24.1,832          | 18                    | 5                |
| 2          | MLCK KD   | 1         | 0.684,661,444     | 32                    | 0                |
| 2          | MLCK KD   | 1         | 93.06,505,201     | 31                    | 29               |
| 2          | MLCK KD   | 1         | 252.6,404,659     | 38                    | 38               |
| 2          | MLCK KD   | 1         | 4.03,461          | 9                     | 8                |
| 2          | MLCK KD   | 1         | 34.35,333,318     | 22                    | 15               |
| 2          | MLCK KD   | 1         | 93.87,201,628     | 11                    | 10               |
| 2          | MLCK KD   | 1         | 166.4,216,827     | 46                    | 40               |
| 2          | MLCK KD   | 1         | 64.18,701,038     | 33                    | 23               |
| 2          | MLCK KD   | 1         | 263.6,191,082     | 94                    | 83               |
| 2          | MLCK KD   | 1         | 5.844,074,469     | 18                    | 2                |
| 2          | MLCK KD   | 1         | 79.7,142          | 17                    | 7                |
| 2          | MLCK KD   | 1         | 203.5,156,143     | 40                    | 34               |
| 2          | MLCK KD   | 1         | 11.27,246,165     | 35                    | 6                |
| 2          | MLCK KD   | 1         | 17.63,003,219     | 7                     | 5                |
| 2          | MLCK KD   | 2         | 162.411           | 27                    | 19               |
| 2          | MLCK KD   | 2         | 1.34,487          | 7                     | 0                |
| 2          | MLCK KD   | 2         | 36.53,157,848     | 32                    | 12               |
| 2          | MLCK KD   | 2         | 0                 | 33                    | 0                |
| 2          | MLCK KD   | 2         | 94.16,540,076     | 63                    | 48               |
| 2          | MLCK KD   | 2         | 4.376,942,803     | 7                     | 0                |
| 2          | MLCK KD   | 2         | 13.8,888          | 48                    | 2                |
| 2          | MLCK KD   | 2         | 0                 | 18                    | 0                |
| 2          | MLCK KD   | 2         | 0                 | 32                    | 0                |
| 2          | MLCK KD   | 2         | 6.944,423,219     | 66                    | 3                |
| 2          | MLCK KD   | 2         | 10.24,546,947     | 32                    | 7                |
| 2          | MLCK KD   | 2         | 190.4,583,442     | 38                    | 34               |
| 2          | MLCK KD   | 2         | 119.8,891         | 31                    | 10               |
| 2          | MLCK KD   | 2         | 77.12,222,124     | 34                    | 27               |
| 2          | MLCK KD   | 2         | 4.35,429          | 9                     | 2                |
| 2          | MLCK KD   | 2         | 126.8,823,909     | 38                    | 32               |
| 2          | MLCK KD   | 2         | 44.6,415,923      | 21                    | 7                |
| 2          | MLCK KD   | 2         | 114.4,104         | 62                    | 46               |
| 2          | MLCK KD   | 2         | 36.8,006          | 23                    | 5                |
| 2          | MLCK KD   | 2         | 180.3,349,339     | 26                    | 22               |
| 2          | MLCK KD   | 2         | 116.090,192       | 32                    | 27               |
| 2          | MLCK KD   | 2         | 155.320,339       | 34                    | 26               |
| 2          | MLCK KD   | 2         | 41.2,508          | 44                    | 8                |
| 3          | MLCK KD   | 1         | 0                 | 14                    | 0                |
| 3          | MLCK KD   | 1         | 121.0,383,624     | 28                    | 24               |
| 3          | MLCK KD   | 1         | 12.69,068,891     | 18                    | 2                |
| 3          | MLCK KD   | 1         | 3.12,988          | 3                     | 0                |
| 3          | MLCK KD   | 1         | 8.876,141,581     | 22                    | 4                |
| 3          | MLCK KD   | 1         | 8.411,333,388     | 24                    | 3                |
| 3          | MLCK KD   | 1         | 0                 | 0                     | 0                |
| 3          | MLCK KD   | 1         | 0                 | 7                     | 0                |
| 3          | MLCK KD   | 1         | 0                 | 10                    | 0                |
| 3          | MLCK KD   | 1         | 19.61,065,993     | 19                    | 6                |
| 3          | MLCK KD   | 1         | 10.41,663,483     | 30                    | 6                |
| 3          | MLCK KD   | 1         | 52.49,886,144     | 21                    | 20               |
| 3          | MLCK KD   | 1         | 5.403,934,969     | 17                    | 1                |


Table 1 (continued)

| Experiment | Condition | Replicate | Degradation (μm²) | Degrading Invadopodia | Total Invadopodia |
|------------|-----------|-----------|-------------------|-----------------------|------------------|
| 3          | MLCK KD   | 1         | 1625.948,669      | 0                     | 0                |
| 3          | MLCK KD   | 1         | 41.32,420,742     | 11                    | 10               |
| 3          | MLCK KD   | 1         | 19.14,606,869     | 20                    | 17               |
| 3          | MLCK KD   | 1         | 11.12,574,847     | 26                    | 7                |
| 3          | MLCK KD   | 1         | 37.92,536,432     | 24                    | 15               |

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