1 Dasatinib affects focal adhesion and myosin regulation to inhibit matrix contraction by
2 Müller cells

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Abstract

Epiretinal membrane (ERM) contraction is associated with a variety of ocular diseases that cause macular dysfunction. Trans-differentiated Müller cells have been identified in ERMs, and have been implicated to be involved in the contractile process. In this study, we tested the effect of dasatinib, a FDA-approved tyrosine kinase inhibitor, on matrix contraction caused by Müller cells, and examined molecular mechanism of action. Type I collagen matrix contraction assays were used to examine the effect of drugs on matrix contraction by trans-differentiated Müller cells. Fluophore-conjugated phalloidin was used for the detection of actin cytoskeleton, and Western-blot analyses were carried out to examine protein expression and phosphorylation status. Dasatinib inhibited collagen matrix contraction by trans-differentiated Müller cells that was associated with decreased cell spreading and reduction of actomyosin stress fibers. Concomitantly, dasatinib-treated Müller cells had reduced phosphorylation of Src family kinase, paxillin, as well as myosin II light chain. Specific inhibitors of Rho/ROCK and myosin II confirmed the critical role played by this pathway in Müller cell contraction. Our data demonstrate that dasatinib significantly reduced matrix contraction by Müller cells via

1 inhibition of focal adhesion, as well as actomyosin contraction.

2

1. Introduction

The formation and contraction of epiretinal membranes (ERMs), an avascular fibrotic scar tissue on the retinal surface, causes macular dysfunction associated with a variety of ocular diseases including proliferative retinopathies, macular holes and puckers (Bu et al., 2014a; Messmer et al., 1998; Pournaras et al., 2000; Ryan, 1985; Snead et al., 2008).

Tension on the retina created by ERM contraction can result in metamorphopsia, reduced visual acuity, and even partial loss of vision (from traction retinal detachment - TRD). While preventing the formation of ERMs should be considered the first line of defense, inhibiting the contraction of these membranes once they form becomes critical to minimize the adverse effect on vision. Therefore, it is important to identify drugs that can prevent contraction, as well as to understand the molecular mechanisms involved in the contractile process.

Glial cells have been long identified as a major cell type in idiopathic ERMs (Foos, 1974; Kampik et al., 1980). Recent studies have identified trans-differentiated Müller cells, which express marker proteins for fibroblasts and/or myofibroblasts in addition to glial cell markers, to be the major cell types in ERMs associated with macular holes

and pucker (Bu et al., 2014b; Zhao et al., 2013). Additionally, trans-differentiated Müller cells have been identified in ERMs from patients with proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathies (PDR) (Feist et al., 2014; Guidry et al., 2009). Gliotic trans-differentiation of Müller cells has been implicated following retinal damage or chronic inflammation (Bringmann et al., 2006; Bringmann and Wiedemann, 2009). Furthermore, Guidry and colleagues have demonstrated that Müller cells can trans-differentiate to adopt fibroblast and myofibroblast phenotypes upon culture in vitro, and while freshly isolated Müller cells are not contractile, trans-differentiated Müller cells can exert strong contractile forces (Guidry, 1996, 1997; Guidry et al., 2003). Thus, available data strongly suggests that trans-differentiated Müller cells contribute to the contractile force exerted by ERMs.

We have previously demonstrated that dasatinib, a FDA-approved tyrosine kinase inhibitor, can inhibit TRD in a swine model of PVR, and that dasatinib was inhibiting cell contraction of de-differentiated retinal pigment epithelial (RPE) cells in the PVR model (Umazume et al., 2013). Other studies have shown, however, that trans-differentiated Müller cells can exert stronger contractile

force than trans-differentiated RPE cells (Guidry et al., 1992). In this study, we investigated if dasatinib can inhibit matrix contraction by trans-differentiated Müller cells, and examined the molecular mechanisms of the contractile process targeted by the drug.

2. Materials and Methods

2.1. Isolation and culture of porcine Müller cells

The use of animal tissue was approved by the University of Louisville Institutional Animal Care and Use Committee and adhered to the ARVO animal Statement for the Use of Animals in Ophthalmic and Vision Research.

Müller cells and vitreous were prepared from fresh porcine eyes obtained from Swift Meat Packing Co. (Louisville, KY). Porcine Müller cells were isolated based on previously reported methods (Guidry, 1996; Hauck et al., 2003). Briefly, following several washes with Dulbecco's phosphate buffered saline (DPBS) and removal of major blood vessels, retinæ were digested using papain/DNase (Papain Dissociation System, Worthington Biochemicals, Lakewood, NJ) at 37°C for 60 minutes. After neutralizing with

albumin-ovomucoid, retinal cells were dissociated by repeated trituration and the cell suspension was allowed to settle for 10 minutes. The supernatant was collected, spun at 300x g for 6 minutes, and resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were allowed to attach on collagen I coated flasks for 30 minutes, after which the flasks were gently rinsed with DPBS. Cells remaining on the flask were cultured in 10%-FBS-DMEM. Upon confluence, cells were subcultured with 0.05% trypsin/EDTA, and used for experiments between passage 3 and 5. As noted in previous studies(Guidry, 1996; Hauck et al., 2003), cells isolated and cultured by this method yielded high percentage (>95%) of cells positive for Müller cells markers glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) during early passages, however, the signal for both proteins became progressively weaker with increasing culture time and passage.

2.2.Collagen matrix contraction assay

Matrix contraction was quantified by adapting an established collagen matrix contraction assay for RPE cells(Morales et al., 2007; Umazume et al., 2014). To each well

containing 0.4ml of solidified neutralized type I collagen (2.5 mg/mL, Corning, Tewksbury, MA), 2×10^5 of Müller cells in 25% vitreous fluid-DMEM were added. Porcine vitreous fluid was prepared as previously reported (Meitinger et al., 2001; Umazume et al., 2013). Effects of the following drugs on matrix contraction were examined: 0.3 μ M dasatinib (LC Laboratories, Woburn, MA), 5 μ M Y-27632 dihydrochloride (Tocris Bioscience, Bristol, UK), 20 μ M (+)-Blebbistatin or (-)-Blebbistatin (Calbiochem, La Jolla, CA). The drugs were dissolved in dimethyl sulfoxide (DMSO), which was used as a control. Drugs were administered following 2 days of culture on collagen matrices. One day after drug administration (a total of 3 days of culture), the matrices were gently released from wells and photographed after 4 hours. The area of the matrix was quantified using Photoshop CS6 software version 13.0.6 (Adobe Systems, San Jose, CA), and the degree of contraction, relative to the size of the well, was determined. Results are mean $\pm$ SEM of 9 different samples for each condition from three different batches, and the data is normalized to control condition for each batch.

2.3.Fluorescent Staining

1 *2.3.1. Proliferation assay using EdU uptake*

2 Müller cells were cultured on collagen I matrices for a total of three days in 25% vitreous
3 fluid-DMEM in the presence or absence of dasatinib (0.3 µM) for the final 24 hours.

4 DMSO (0.1%) treated cells served as controls. Cell proliferation was examined using a
5 commercially available kit (Click-iT® EdU Alexa Fluor® 488 Imaging Kit, Life

6 Technologies, Grand Island, NY) and followed the manufacturer's protocol. In short,

7 10µM EdU was added to the cultured media for the final 4 hours of culture. Following

8 fixation with 4% paraformaldehyde for 15 minutes and three washes with 3%BSA-PBS,

9 cells were permeabilized with 0.5% Triton® X-100 in PBS for 20 minutes. After two

10 washes, cells were incubated with Click-iT® reaction cocktail that contains the

11 AlexaFluor 488 dye for 30 minutes, washed once, nucleus counterstained using Hoechst

12 33342, and observed under a fluorescent microscope. Data from three different

13 experiments with duplicates for each experiment was used to determine the percentage

14 of EdU positive cells by the following equation

15
$$(\text{EdU positive nuclei}) / (\text{total number of nuclei}) \times 100$$

16 *2.3.2. TUNEL assay for apoptosis*

Müller cells were cultured on collagen I matrices for a total of three days in 25% vitreous fluid-DMEM in the presence or absence of dasatinib (0.3 μ M) for the final 24 hours. DMSO (0.1%) treated cells served as controls. Apoptosis was assessed using a commercially available TUNEL assay kit (Dead End Fluorometric TUNEL System, Promega, Madison, WI) as previously reported (Umazume et al., 2013). Data from three different experiments with triplicates for each experiment was used to determine the percentage of apoptotic cells by the following equation

$$(\text{TUNEL positive nuclei}) / (\text{total number of nuclei}) \times 100$$

2.3.3. Staining for F-actin

DMSO (0.1%, used as a control) or dasatinib (0.3 μ M) was added to the media of Müller cells cultured on collagen I matrices for two days in 25% vitreous fluid-DMEM, and cells were fixed after 2, 4, 6, 12 or 24 hours with 4% paraformaldehyde. After three washes with PBS, cytoskeletal actin was stained using Alexa Fluor 488-conjugated phalloidin (Life Technologies). Following three washes with PBS, samples were mounted with Vectashield with DAPI (H-1200, Vector Laboratories, Burlingame, CA), and observed using a fluorescence microscope (Axiovert 200, Zeiss Microimaging, Thornwood, NY).

2.4. Immunoblot Analysis

Müller cells were seeded on collagen I-coated 35mm dish, and cultured for 3 days in 25% vitreous fluid-DMEM in the presence or absence of dasatinib for the final 24 hours. Cells were lysed in RIPA buffer composed of 50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 1.6mM Na orthovanadate, 500mM Na fluoride and protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO). Protein concentration was determined by BCA assay (Pierce, Rockford, IL). Western blot analyses were performed as described previously using Odyssey infrared scanner (LI-COR Biosciences, Lincoln, NE) for detection of infrared dye-conjugated secondary antibodies.(Tamiya et al., 2010)Primary antibodies (Ab) used were rabbit monoclonal anti-PY416-SFK Ab, rabbit polyclonal anti-PY118-paxillin Ab, rabbit monoclonal PDGFR α Ab, rabbit monoclonal anti-PY1059-VEGFR2 Ab, goat polyclonal anti-VEGFR2 Ab, mouse monoclonal anti-phospho-Myosin Light Chain (MLC)2 Ab, rabbit polyclonal anti-MLC2 Ab, rabbit monoclonal anti- β -actin Ab (all from Cell Signaling Technology, Danvers, MA), mouse monoclonal paxillin Ab (Santa Cruz Biotech,

Santa Cruz, CA), and mouse monoclonal anti- α -smooth muscle actin (α -SMA) Ab (Sigma Aldrich, St. Louis, MO). The data is normalized to control condition (treated with DMSO), and results are mean $\pm$ SEM of 3 different samples.

2.5. Statistical Analysis

Student's *t*-test was used for analyses of collagen matrix contraction, proliferation, apoptosis, and immunoblot assays. MedCalc statistical software version 12.1.1 (MedCalc Software, Ostend, Belgium) was used for statistical analysis. A *P* value of < 0.05 was considered statistically significant.

3. Results

3.1. Effect of Dasatinib on Matrix contraction by Müller cells

In agreement with previous reports (Guidry, 1997; Hauck et al., 2003), porcine Müller cells adopted a fibroblastic/myofibroblastic morphology in culture over time, accompanied by expression of α -SMA (data now shown). When plated on collagen matrix, these trans-differentiated Müller cells, upon stimulation with vitreous fluid (25% v/v),

effectively contracted collagen matrices (Fig.1A&C). Non-stimulated cells in DMEM alone failed to contract collagen matrices (data not shown). We investigated if 0.3 μ M dasatinib, which does not cause any detectable toxic effect on swine retina following repeated intravitreal injection (Umazume et al., 2013), could inhibit matrix contraction by trans-differentiated Müller cells. In the presence of dasatinib, collagen matrix contraction was inhibited significantly (Fig 1B&C).

3.2. Effects of dasatinib on cell proliferation and apoptosis

As potential mechanisms for the inhibition of matrix contraction by dasatinib, effects of dasatinib on cell proliferation and apoptosis were examined. Little Müller cell proliferation was observed on collagen matrices, and exposure to dasatinib had no significant effect (Fig.2A). Furthermore, dasatinib did not significantly increase the percentage of apoptotic cells (Fig.2B). Therefore, the inhibitory effect of dasatinib on collagen matrix contraction was not due to changes in proliferation or apoptosis.

3.3. Dasatinib affects cell morphology and actomyosin stress fibers

1 To elucidate the mechanism underlying the inhibitory effect of dasatinib on matrix
2 contraction by trans-differentiated Müller cells, we initially examined the effect of the
3 drug on cellular morphology. Under control condition, trans-differentiated Müller cells
4 had a well-spread, elongated morphology with strong expression of actomyosin stress
5 fibers (Fig.3, left panel). Dasatinib treatment induced gradual morphological
6 alteration over time, with drastic changes observed at >12 hours with cells becoming less
7 spread and stress fibers decreasing in number (Fig.3).

9 *3.4. Dasatinib alters phosphorylation status of focal adhesion proteins*

10 Reduced cell spreading implies weaker cell-matrix adhesion. Therefore, we examined the
11 effect of dasatinib on focal adhesion (FA) proteins, in particular phosphorylation status of
12 two key proteins within the FA complex, by Western blot analyses. Dasatinib treatment
13 significantly reduced the expression of active Src family kinases (SFKs), which are
14 phosphorylated on tyrosine 416 (PY416-SFKs) (Fig 4A, C). Furthermore, dasatinib
15 treatment significantly reduced phosphorylation of paxillin, a key FA adaptor protein
16 and a major SFK phosphorylation target (Deakin and Turner, 2008) (Fig 4B, C).

3.5. Effect of dasatinib on the proteins involved in contraction

The effect of dasatinib on receptor tyrosine kinases PDGF receptor α (PDGFR α) and VEGF receptor 2 (VEGFR2), both of which have been implicated to play a role in vitreoretinal fibroproliferative diseases as well as in cell contraction (Kinose et al., 2005; Pennock et al., 2014; Yang et al., 2011), were examined. PDGFR α expression was below detection in both control and dasatinib-treated cells (results not shown). Dasatinib had no significant effect on the expression of active VEGFR2, phosphorylated on the major auto-phosphorylation site tyrosine 1059 within the tyrosine kinase catalytic domain (Fig. 5A).

The actomyosin complex plays a critical role in cellular contraction, and dasatinib clearly affects the contractile process as evidenced by the reduction of actomyosin stress fibers (Fig. 3). We further examined the effect of dasatinib on key elements of the contractile apparatus by Western blot analyses. In trans-differentiated Müller cells treated with dasatinib expression of phosphorylated MLC2 decreased significantly while total MLC2 expression was not affected (Fig 5B); thus, the ratio of phospho-/total-MLC

1 significantly decreased (Fig 5D). In contrast, expression of α -SMA, an actin isoform
2 reported to exert stronger contraction than β - or γ -actin (Hinz et al., 2001), was not
3 affected by dasatinib treatment (Fig 5C, D).

4 5 *3.6. Effect of inhibitors of ROCK and myosin on Matrix contraction by Müller cells*

6 As dasatinib significantly reduced the phosphorylation of MLC2, we examined the role of
7 myosin II, the main target of MLC2 in matrix contraction by Müller cells. In addition, we
8 also examined the effect of an inhibitor of ROCK, which is an upstream regulator of
9 MLC2, on matrix contraction by trans-differentiated Müller cells. Treatment of cells
10 with (-)-Blebbistatin, a myosin II inhibitor (Straight et al., 2003), significantly inhibited
11 matrix contraction compared to the control (+)-Blebbistatin, the inactive enantiomer
12 (Fig 6A). Furthermore, treatment of cells with Y-27632, a ROCK inhibitor (Uehata et al.,
13 1997), significantly inhibited matrix contraction (Fig 6B).

14 15 **Discussion**

16 In the current study, we have investigated the effect of dasatinib on matrix contraction

1 bytrans-differentiated Müller cells.We utilized the stressed matrix contraction assay
2 that develops a high cell-matrix tension state(Grinnell and Petroll, 2010) since we
3 believe a similar build up intension is caused by ERMs. Our results showed that
4 dasatinib can effectively inhibit collagen matrix contraction bytrans-differentiated
5 Müller cells at a submicromolar concentration. This concentration is comparable to the
6 plasma concentration achieved in patients following oral drug intake(Christopher et al.,
7 2008), and we have previously demonstrated that multiple intravitreal injection of
8 0.3 μ M dasatinib has no detectable toxic side-effects in swine eyes(Umazume et al.,
9 2013).

10 In the stressed matrix contraction assay, cellular tension exerted by actomyosin
11 contraction leads to prominent stress fiber formation and increased cell adhesion by
12 maturation of FAs, which transmit cellular contractility to the extracellular matrix
13 (ECM) by tethering the actin cytoskeleton to the ECM (Fig.7)(Burridge and
14 Chrzanowska-Wodnicka, 1996; Dumbauld et al., 2010; Grinnell and Petroll,
15 2010).Dasatinib treatment resulted in reduction ofboth cell spreading, as well as stress
16 fiber formation. This was accompanied by reduced phosphorylation on tyrosine residues

416 and 118 of SFKs and paxillin, respectively. SFKs play a regulatory role by phosphorylating proteins within the FA complex(Thomas and Brugge, 1997).Src itself is autophosphorylated on tyrosine 416 to fully activate kinase function(Thomas and Brugge, 1997).Similar to our finding in the current study, inhibition of SFK autophosphorylation by dasatinibhas been accompanied by reduced cell spreading in other cell types(Caccia et al., 2010; Pichot et al., 2009).Additionally, phosphorylation of paxillin, a key adaptor protein within FAs, plays an important role in cell adhesion, migration, and contraction(Deakin and Turner, 2008).Past studies have shown that paxillin is phosphorylated under conditions in which cell adhesion is strengthened, such asduring cell contraction, migration, and/or FA maturation under tension(Chrzanowska-Wodnicka and Burridge, 1996; Pasapera et al., 2010; Schneider et al., 2009).Thus, the observed reduction of PY118-paxillin by dasatinib in this study implicatesweakenedcell adhesion to the ECMthat may contribute to reduced cell spreading. Furthermore, weaker adhesion should result in less contraction as tension applied to the matrix will also be reduced.

We also examined the effect of dasatinib on the actomyosin contractile apparatus.

1 It is well established that activation of Rho and its downstream effector ROCK targets
2 phosphorylation of MLC, which results in activation of myosin, stress fiber assembly,
3 and increased actomyosin contraction (Fig.7)(Amano et al., 1997; Burridge and Wittchen,
4 2013; Riento and Ridley, 2003).Dasatinib inhibited actomyosin contraction as evidenced
5 by significant reduction in MLC phosphorylation. We confirmed the importance of
6 myosin II and ROCK activity in matrix contraction using specific inhibitors
7 (-)-blebbistatin (for myosin II)and Y-27632 (for ROCK). Rho is activated by Rho Guanine
8 nucleotide exchange factors (GEFs), and a recent study revealed that SFK member Fyn
9 is involved in Rho activation in cells under tension via leukemia-associated Rho GEF
10 (LARG)(Guilluy et al., 2011).Thus, the observed reduced MLC phosphorylation by
11 dasatinib, a SFK inhibitor, could be via prevention of LARG activation by Fyn resulting
12 in inhibition of Rho activation.

13 Aberrant and sustained activation of myofibroblasts, which are involved in
14 fibrotic ECM accumulation as well as contraction of the fibrotic matrix, have been
15 implicated in a variety of fibrotic disorders(Hinz et al., 2012).Alpha-SMA is an actin
16 isoform that has stronger contractility than other actin isoforms(Hinz et al., 2001). It is

1 highly expressed in myofibroblasts, and thus, is widely used as a marker protein for this
2 cell phenotype. Dasatinib had no significant effect on α -SMA expression, and therefore,
3 its inhibitory effect on contraction is not via a change in actin isoforms.

4 In summary, we have demonstrated that the tyrosine kinase inhibitor dasatinib
5 can inhibit collagen matrix contraction by trans-differentiated Müller cells at
6 submicromolar concentrations. The inhibitory effect of dasatinib on contraction is via
7 reduced cell-ECM adhesion, as well as inhibition of the actomyosin contractile
8 apparatus. Trans-differentiated Müller cells have been implicated in ERMs associated
9 with macular holes, macular pucker, PVR and PDR (Bu et al., 2014b; Feist et al., 2014;
10 Guidry et al., 2009; Zhao et al., 2013), and therefore, further studies may be warranted to
11 examine the potential therapeutic role of dasatinib in these diseases.

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Figure captions

Figure 1. Dasatinib inhibits collagen matrix contraction by trans-differentiated Müller cells. Representative images of contracted collagen matrix (A) under control condition (without dasatinib) or (B) in the presence of dasatinib are shown. Dotted black lines indicate the size of collagen matrix prior to release, while the solid blue lines show the edge of the collagen matrix following contraction. (C) Quantitative analysis of collagen matrix contraction. Dasatinib significantly inhibited collagen matrix contraction.

* $P < 0.05$, significantly different from control.

Figure 2. Dasatinib has no significant effect on proliferation and apoptosis of trans-differentiated Müller cells. (A) Effect of dasatinib on proliferation. EdU uptake reveals minimal proliferation on collagen matrices with no significant difference between dasatinib and control. The data are from three different experiments with duplicates for each experiment. (B) Effect of dasatinib on apoptosis. No significant difference was observed between dasatinib and control. Data expressed as means $\pm$ SEM of three different samples.

Figure 3. Dasatinib induce morphological changes to trans-differentiated Müller cells.

Following two days of culture, dasatinib was added to the culture media, and cells were fixed after 2, 4, 6, 12 or 24 hours, and stained for filamentous actin with Alexa-488 conjugated phalloidin (green). Dasatinib reduced both cell spreading as well as actin stress fiber expression with the changes becoming more severe over time. Scale bar, 100 μm .

Figure 4. Dasatinib reduces the tyrosine phosphorylation of SFKs and paxillin.

Representative Western blot images for (A) active SFKs (PY416-SFK) and (B) phospho-paxillin (PY118-Pax) together with their loading control used for normalization, β -actin and total paxillin respectively, are shown. (C) Quantified data for normalized Western blot band densities of active SFKs and phospho-paxillin. Dasatinib significantly reduced tyrosine phosphorylation of each protein. * $P < 0.05$, significantly different from control.

Figure 5. Effects of dasatinib on RTKs involved vitreoretinal fibrosis as well as contractile proteins. Representative Western blot images for (A) phospho- and total VEGFR2, (B) phospho- and total MLC and (C) α -SMA and β -actin of trans-differentiated Müller cells are shown. (A) Dasatinib had no significant effect on the major autophosphorylation site Tyr1059 of VEGFR2. (B) Dasatinib significantly reduced MLC phosphorylation. (C) Dasatinib had no significant effect on α -SMA expression. (D) Quantified data for Western blot band densities clearly shows reduction in phospho-MLC by dasatinib, while phospho-VEGFR2 and α -SMA expression are not affected. * $P < 0.05$, significantly different from control.

Figure 6. Myosin II inhibitor (-)-Blebbistatin and ROCK inhibitor Y-27632 reduces matrix contraction by trans-differentiated Müller cells. (A) The effect of (-)-Blebbistatin (20 μ M) was compared to its inactive enantiomer (+)-Blebbistatin (20 μ M). The data is normalized to (+)-Blebbistatin, and results show significant inhibition of contraction by (-)-Blebbistatin. (B) Y-27632 (5 μ M) significantly inhibited contraction. * $P < 0.05$, significantly different from control.

Figure 7. A model for the mechanism of action by dasatinib to prevent matrix contraction.

(A) Molecular mechanisms involved in matrix contraction. Cell adhesion and integrin ligation activate SFKs within the focal adhesion (FA) complex (Thomas and Brugge, 1997). This results in SFK auto-phosphorylation as well as phosphorylation of FA adaptor protein paxillin, which is required for the maturation of FAs (Pasapera et al., 2010). Increase in cell-matrix tension activates Rho via the activation of leukemia-associated Rho GEF (LARG) by SFK member Fyn (Guilluy et al., 2011). Stimulation of myosin activity leads to assembly of stress fibers as well as actomyosin contractility. Concomitantly, cell-matrix tension also induces FA maturation resulting in stronger cell-matrix adhesion. Contraction of dense stress fibers supported by strong cell-ECM adhesion is necessary for matrix contraction.

(B) Under control condition (without dasatinib), well-spread cells with dense stress fibers and strong cell-ECM adhesion exert strong contractile force on the ECM. In the presence of dasatinib, cell spreading is reduced (implying weaker cell-ECM adhesion), stress fibers are diminished and the cells become weakly contractile.

Figure 3
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Figure 7
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