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ORIGINAL PAPER

Increased CCT-eta expression is a marker of latent and active disease and a modulator of fibroblast contractility in Dupuytren's contracture

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Abstract Dupuytren's contracture (DC) is a fibroproliferative disorder of unknown etiology characterized by a scar-like contracture that develops in the palm and/or digits. We have previously reported that the eta subunit of the chaperonin containing T-complex polypeptide (CCT-eta) is increased in fibrotic wound healing, and is essential for the accumulation of α-smooth muscle actin (α-SMA) in fibroblasts. The purpose of this study was to determine if CCT-eta is similarly implicated in the aberrant fibrosis seen in DC and to investigate the role of CCT-eta in the behavior of myo/fibroblasts in DC. Fibroblasts were obtained from DC-affected palmar fascia, from adjacent phenotypically normal palmar fascia in the same DC patients (PF), and from non-DC palmar fascial tissues in patients undergoing carpal tunnel (CT) release. Inherent contractility in these three populations was examined using fibroblast-populated collagen lattices (FPCLs) and by cell traction force microscopy. Expression of CCT-eta and α-SMA protein was determined by Western blot. The effect of CCT-eta inhibition on the contractility of DC cells was determined by deploying an siRNA versus CCT-eta. DC cells were significantly more contractile than both matching palmar fascial (PF) cells and CT cells in both assays, with PF cells demonstrating an intermediate contractility in the FPCL assay. Whereas α-SMA protein was significantly increased only in DC cells compared to PF and CT cells, CCT-eta protein was significantly increased in both PF and DC cells compared to CT cells. siRNA-mediated depletion of CCT-eta inhibited the accumulation of both CCT-eta and α-SMA protein in DC cells, and also significantly decreased the contractility of treated DC cells. These observations suggest that increased expression of CCT-eta appears to be a marker for latent and active disease in these patients and to be essential for the increased contractility exhibited by these fibroblasts.

Keywords Dupuytren's contracture · Palmar fascia · Fibroblast · Contractility · Traction force · CCT-eta · α-Smooth muscle actin

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Introduction

Dupuytren's contracture (DC) is a common progressive fibroproliferative disorder that manifests in the fascia of the palm and digits (the "palmar fascia") resulting in deformities and impairment of hand function (Hindocha et al. 2008). The ring and small fingers are most often involved, and the disease is thought to progress from a palmar nodule into a longitudinal cord. As the cord matures, it contracts and becomes more fibrotic, with resulting flexion contractures of the digits (McFarlane 1974; Shaw et al. 2007). The disease is more

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prevalent in men than in women with a male to female ratio of between 5:1 and 15:1 (Degreef et al. 2008), and a genetic predisposition to disease development is clearly evident in some families (Mcfarlane 2002; Michou et al. 2012).

The mainstay of treatment for DC remains surgical excision of the diseased tissue, but this is not without substantial risks, including damage to the digital nerves and blood vessels, damage to the underlying flexor tendons, and the possibility of skin necrosis. In addition, DC recurrence rates after surgery range from 27 % to 80 % (Rodrigo et al. 1976; Tonkin et al. 1985; Makela et al. 1991), leading some patients to opt for finger amputation to eliminate the debilitating contractures (Kobus et al. 2007). Numerous nonsurgical treatment options, including corticosteroid injections, vitamin A and E application, radiotherapy, dimethylsulfoxide injections, ultrasound therapy, 5-fluorouracil treatment, and gamma interferon injections have failed to gain clinical currency (Hurst and Badalamente 1999).

Recently, injection of a mixture of clostridium-derived collagenases has been proposed as a safe and effective nonoperative intervention for the treatment of diseased cords to improve finger contractures and joint mobility in advanced disease (Badalamente et al. 2002; Badalamente and Hurst 2007; Hurst et al. 2009; Watt et al. 2010). While the long-term safety profile and recurrence rate of this therapy is still unclear, a recent study reported flexor tendon ruptures in two patients, indicating the potential for serious problems (Zhang et al. 2011). There remains a pressing need for a better understanding of the biology of DC progression and recurrence, and novel therapeutic targets to address the same.

The hallmark of DC disease is fibroblast proliferation and myofibroblast differentiation; DC is characterized by the persistence of myofibroblasts within lesions, and these cells are thought to be the principal effectors of the disease phenotype (Verjee et al. 2009, 2010). Myofibroblasts are typically recognized by their characteristic expression of α-smooth muscle actin (α-SMA), which has been implicated in both cell motility and its ability to exert a deforming contractile force on surrounding tissues (Hinz 2007). Targeting molecules that enable myofibroblast function is therefore an attractive option to potentially mitigate the progression or recurrence of DC disease at a cellular level.

We have previously observed that the expression of the eta subunit of the chaperonin containing T-complex polypeptide (CCT-eta) is decreased during fetal skin wound healing (which occurs without fibrosis, without contracture, and without myofibroblasts) and increased in adult skin wounds (which do heal with scar, with contracture, and with myofibroblasts). This pattern of expression is not shared by any other CCT subunit (Satish et al. 2008, 2010a, b). The chaperonin containing T-complex polypeptide is a hetero-oligomeric particle with eight distinct subunits that is thought to assist in the folding of up to 15 % of cellular proteins, including major cytoskeletal proteins such as tubulin and actin (Kubota et al. 1994, 1995). We have previously shown that siRNA-mediated reduction of CCT-eta (but not its closest evolutionary homolog CCT-beta) results in decreased α-SMA expression as well as decreased contractility and motility in skin-derived fibroblasts. We have now investigated the role of CCT-eta in the behavior of myo/fibroblasts in DC. In the present study, we also sought to compare the contractile properties of three distinct cell populations: fibroblasts from areas of active DC disease (DC), from patient-matched clinically normal palmar fascia (PF), and from non-DC palmar fascia in patients undergoing carpal tunnel (CT) release.

Materials and methods

Cell culture

DC cord samples from six patients and small samples of phenotypically normal palmar fascia tissue (PF) from the same patients were surgically explanted at the Hand and Upper Limb Centre at St. Joseph's Health Care (SJHC), London, ON, Canada. We also obtained phenotypically normal palmar fascia from six patients undergoing CT release at SJHC as previously described (Howard et al. 2003; Satish et al. 2011). All samples were collected with the informed consent of the patient and with Institutional Review Board approval, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Fibroblasts were obtained by outgrowth in cultures in MEM-α medium (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (FBS, Gemini Bioproducts, West Sacramento, CA, USA) and 1 % antibiotic-antimycotic solution (Sigma, St. Louis, MO, USA). All cultures were used at passage levels between 2 and 6 with no aberrant changes evident in cell morphology.

Fibroblast-populated collagen lattice (FPCL) contraction assay

A widely used assay to test cell contractility is the fibroblast-populated collagen lattice (FPCL) assay (Bell et al. 1979). Collagen contraction was carried out using disease fibroblasts from DC-affected palmar fascia (DC), fibroblasts from uninvolved palmar fascia of DC patients (PF), and fibroblasts derived from carpal tunnel release patients without DC (CT). Collagen lattices were prepared by mixing cell suspensions with a neutralized solution of type I collagen matrix (4 parts rat tail collagen, 1 part neutralization solution; 2 parts 0.34 M NaOH:3 parts 10X Waymouth media). Each well in a 24-well culture dish contained 1×10^5 cells in 0.5 ml of the collagen mixture. Following FPCL polymerization (1 h, 37 °C), culture

medium (0.5 ml) consisting of alpha-MEM +2 % FBS was added to the top of each lattice. The attached FPCLs were then manually released from the sides of the culture plates and digitally scanned 24 h after release. The area of the lattices was quantified using NIH ImageJ. Assays were performed on fibroblasts obtained from three different patients in triplicate.

Cell traction force microscopy

Another means of determining the contractility of cells is by cell traction force microscopy (CTFM) as has been previously described (Butler et al. 2002). The CTFM method computes CTFs based on substrate surface deformations caused by attached cells on a thin elastic substrate (Beningo et al. 2002; Beningo and Wang 2002; Yang et al. 2006). Briefly, polyacrylamide gel disks, 120 μm thick and 10 mm in diameter, were embedded with 0.2 μM green fluorescent microbeads (Molecular Probes, Eugene, OR, USA) and attached to the bottom of a 35-mm glass dish (MatTek, Ashland, MA, USA), which had a 14-mm circular inner glass area and had been consecutively treated with 0.1 M sodium hydroxide, 3-aminopropyltrimethoxysilane, and 0.5 % glutaraldehyde. After polymerization, the gel surface was treated with Sulfo-SANPAH (Pierce, Rockford, IL, USA) and then coated with 200 μl of 100 μg/ml collagen type I overnight at 4 °C.

CT-, PF-, and DC-derived fibroblasts were then plated to the collagen-coated polyacrylamide gel disks at a density of 3,000 cells/disk and were allowed to spread on the gel for 6 h in α-MEM medium. The phase contrast image of individual cells and the image of the embedded fluorescent beads were taken. Next, the cells on the gel disk were trypsinized, and images of the fluorescent beads in the same view and the same *z*-plane were taken. Finally, CTFs were computed using a MATLAB program based on the method described in Butler et al. (2002). For experiments involving siRNA, DC-derived fibroblasts were transfected with CCT-eta siRNA as described below and were also co-transfected with the plasmid pdsRed2-C1 which contains red fluorescent protein (Clontech Laboratories, Inc., Mountain View, CA, USA). The transfection procedures were similar to the method previously described by Satish et al. (2010a). Cells visibly expressing red fluorescence after the period of transfection were selected to measure the traction force as these were cells that were clearly successfully transfected with pdsRed2-C1, and therefore likely to have taken up CCT-eta siRNA as well. At least 15 cells in each experimental group were used to determine their average traction forces. The computational program (MATLAB) can calculate "net contractile moment" and "total strain energy"; however, these may vary with cell shape (e.g., elongated vs. round), with local variations in bead density, etc. Cellular traction forces (CTFs) are directly related to the strength of actin–myosin interactions, and hence CTF in the form of root-square mean (RSM) is reported here as a more appropriate index measurement of cellular contractility that is less subject to the above variabilities.

Cell-based siRNA transfections

Small interfering RNA (siRNA) duplexes against human CCT-eta were purchased from Integrated DNA Technologies (Coralville, IA, USA). The siRNA targeting human CCT-eta is as follows:

> Sense: 5′ rGrArArUrGrArUrUrCrArGrUrGrGrUrGrGrCrUTT 3′
> AntiSense: 5′ rArGrCrCrArCrCrArCrUrGrArArUrCrArUrUrCTT 3′.

An HPLC-purified, duplexed Ambion® negative control siRNA that has no significant sequence similarity to mouse, rat, or human gene sequences was purchased from Invitrogen Corporation (Carlsbad, CA, USA). These siRNAs were transiently transfected to DC-derived fibroblasts, which were then assayed for CCT-eta and α-SMA expression and cellular contractility through cellular traction force microscopy (CTFM).

DC-derived fibroblasts were cultured in MEM-α medium (Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen) and 1 % antibiotic-antimycotic solution (Sigma Chemical Corporation, St. Louis, MO, USA). Transfection of siRNAs was performed with the manufacturer's protocol using Lipofectamine 2000 (Invitrogen). Briefly, 3.75 μl of 20 μM siRNA was mixed with 200 μl of Opti-MEM; 4 μl of Lipofectamine 2000 was diluted into 200 μl of Opti-MEM and incubated at room temperature for 5 min. After the incubation, the diluted Lipofectamine 2000 was combined with the diluted siRNAs and then incubated for an additional 20 min (siRNA sequence targeting CCT-eta and control siRNA were both used at a concentration of 75 pM). A total of 400 μl of siRNA–Lipofectamine 2000 complexes was added to each well of cultured DC-derived fibroblasts at ≈ 90 % confluence in a 6-well plate. After 24 h incubation at 37 °C, the cells were switched to quiescent media (MEM-α medium containing 0.1 % dialyzed FBS along with antibiotics) and left undisturbed for 48 h at 37 °C. After 48 h in quiescent media, cells were subjected to the cellular traction force microscopy. Transfection efficiency was >50 %, and to validate the efficacy of target gene knockdown, cells were analyzed for protein expression by Western blot. The intensity of the protein bands was quantified using NIH ImageJ 1.44p, available in the public domain at http://imagej.nih.gov/ij.

Western blots

Cells were lysed using M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, Rockford, IL, USA), and extracted proteins were subjected to Bradford assay to

determine the total protein concentration. Equal quantities of proteins were separated on SDS-PAGE, transferred to a Whatman™ Protran pure nitrocellulose immobilization membrane (GE Health Care, Piscataway, NJ, USA), and probed with antibodies specific to CCT-eta (Cat No. MCA2179, AbD SeroTec, Raleigh, NC, USA) and α-SMA (Cat No. 7817, Abcam, Cambridge, MA, USA), using GAPDH (Cat No. ab8245, Abcam, Cambridge, MA, USA) as loading control. The membranes were conjugated with HRP-labeled secondary antibody, and signals were detected using SuperSignal® West Femto Trial Kit Prod (Thermo Fisher Scientific).

Statistical analysis

Two different statistical analyses were performed to determine the statistical significance. One-way ANOVA with a post-hoc Turkey HSD test was performed to determine p values. p values less than 0.05 were considered significant. Student's t test was used to statistically validate differences between paired experimental and control groups. A probability (p) value of <0.05 was considered significant.

Results

DC-derived fibroblasts have significantly higher inherent contractility than PF- and CT-derived cells

To compare the innate contractility of CT-, PF-, and DC-derived fibroblasts, we used two methods: in the FPCL assay, the summated effects of a population of cells result in contraction on a macroscopic scale, whereas in CTFM, the contractility of individual cells is measured on a microscopic scale. Both of these assays unequivocally demonstrate that DC-derived fibroblasts are significantly more inherently contractile than either PF- or CT-derived cells (Figs. 1 and 2), with CTFM quantifying an ~40 % greater traction force exerted by DC cells than the other cell types. Even more interestingly, the FPCL assay also suggests that PF cells are actually intermediate in their contractile abilities between control CT cells and DC cells from clinically active disease. While we did not see this exact distinction in the CTFM assay, this may simply be due to differences in the capabilities of the assay and the specific experimental parameters employed.

Expression of CCT-eta is significantly increased in both PF- and DC-derived fibroblasts, whereas expression of α-SMA is increased solely in DC-derived cells

Our previous studies have shown the important role played by CCT subunit eta in differentiating scarless fetal from scirrhous adult skin wound healing, and in regulating fibroblast physiology (Satish et al. 2008, 2010a, b). As CT-, PF-, and DC-derived fibroblasts displayed different contractile phenotypes, we sought to determine their basal levels of CCT-eta and α-SMA protein expression. We determined that CCT-eta protein was significantly elevated in DC-derived fibroblasts derived from six unrelated DC patients compared to cells from six control CT patients (Fig. 3a). Moreover, an increase in CCT-eta expression was evident in the PF-derived fibroblasts from clinically normal fascia.

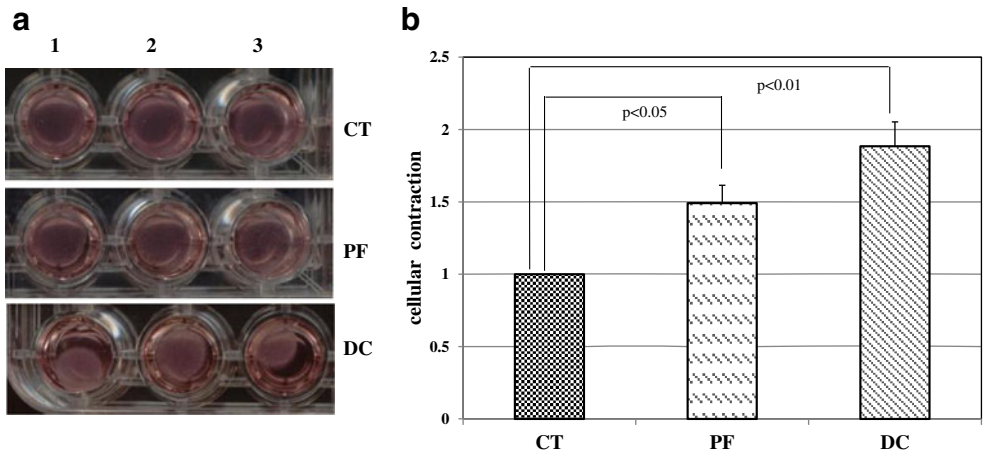

Fig. 1 DC-derived fibroblasts have significantly higher inherent contractility than PF- and CT-derived cells. Collagen lattices seeded with CT-, PF-, and DC-derived fibroblasts obtained from three different patients (*lanes 1–3*) were released manually from the sides of culture plates and digitally scanned 24 h after release. **a** Representative images of collagen lattices seeded with CT-, PF-, and DC-derived fibroblasts 24 h after release. **b** Quantification of gel surface area 24 h after release. Data were obtained using NIH ImageJ to analyze photographic images; data are shown as the area of the contracted collagen lattice normalized to the average area of contraction seen with CT-derived cells, set as a baseline value of 1. Each data point represents the mean±SEM of experiments performed in triplicate from fibroblasts derived from three different patients. Statistical significance was analyzed using one-way ANOVA with a post-hoc Turkey HSD test; p value <0.05 was considered significant

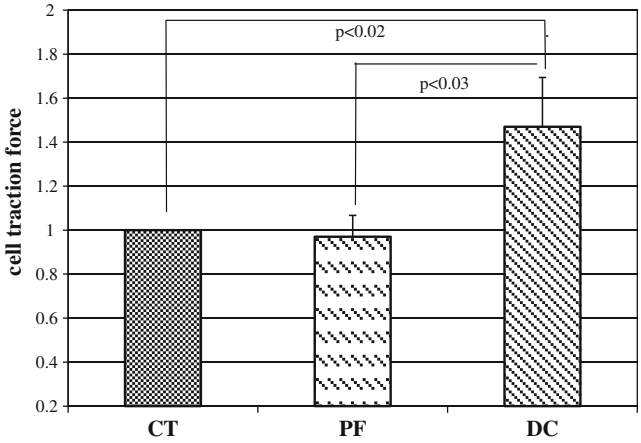

Fig. 2 DC-derived fibroblasts exhibit higher cellular traction force than PF- and CT-derived fibroblasts. Quantification represents the average traction force per cell (±SEM) of more than 15 cells chosen randomly. Results represented here are the experimental data pooled together from three independent experiments. Traction force [expressed as root-square mean (RSM)] exerted by the three cell types was calculated, and the values were normalized to the mean traction force exerted by CT-derived fibroblasts (set to a baseline value of 1). Statistical significance was analyzed using Student's *t* test; *p* value <0.05 was considered significant. There was no statistically significant difference observed in the traction force exerted between CT- and PF-derived fibroblasts, but a significant difference was observed when comparing CT- and DC-derived fibroblasts ($p<0.02$) and comparing PF- and DC-derived fibroblasts ($p<0.03$)

Examination of α-SMA protein levels in these patients revealed that α-SMA expression is consistently higher in DC-derived fibroblasts but, contrary to our findings with CCT-eta, no differences in the accumulation of α-SMA in CT- and PF-derived cells were evident (Fig. 3b). This striking observation suggests that although α-SMA is classically regarded as a marker for the diseased myofibroblast cells seen in active DC, elevated expression CCT-eta may actually be a more sensitive marker that can identify both latent and active disease.

siRNA targeting of CCT-eta markedly inhibits the expression of α-SMA in DC-derived fibroblasts

We have previously shown that reduction of CCT-eta expression in normal adult skin fibroblasts induces a corresponding decrease in the expression of α-SMA (Satish et al. 2010a). We sought to determine if this same pattern would be evident in DC cells. Treatment of DC-derived cells with siRNAs specific for human CCT-eta inhibited accumulation of CCT-eta protein by 39±2 % ($p<0.007$), while treatment with a nonspecific control siRNA has no detectible effect on CCT-eta levels (97±1 %; $p<0.01$; Fig. 4). Assessment of α-SMA protein expression after CCT-eta siRNA application revealed a marked and statistically significant ($p<0.001$) reduction in expression (40.09±2.46 %) compared to basal levels and to the scrambled siRNA control (98±0.17 %; $p<0.001$; Fig. 5).

siRNA-mediated depletion of CCT-eta decreases DC-derived fibroblast contractility

We next sought to determine if reducing CCT-eta levels would affect the contractility of DC-derived fibroblasts. DC cells transfected with CCT-eta or control siRNAs were assayed using CTFM (Fig. 6). Analysis of the displacement fields shows that CCT-eta siRNA specifically decreases DC fibroblast applied traction force by 40 %, approximating the traction force level observed in CT cells, and approximately commensurate with the decrease in α-SMA expression.

Discussion

Since the ultimate manifestation of DC is an actual tissue contracture, the innate contractile ability of fibroblastic cells in DC has been a subject of interest. A study by Tomasek

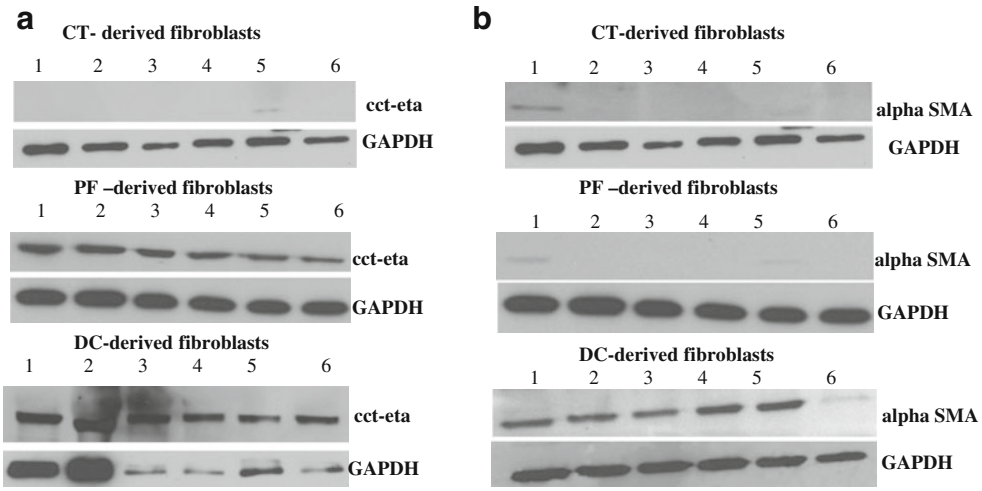

Fig. 3 Expression of CCT-eta and α-SMA protein in CT-, PF-, and DC-derived fibroblasts. Whole cell lysates collected from CT-derived fibroblasts from six carpal tunnel patients, as well as matched PF- and DC-derived fibroblasts from six Dupuytren's patients (*lanes 1–6*) were subjected to Western blot analyses using antibodies specific for CCT-eta (**a**) and α-SMA (**b**) using 20 μg/lane of protein. GAPDH antibody was used as a loading control. Shown here is a representation of two independent experiments with essentially identical results

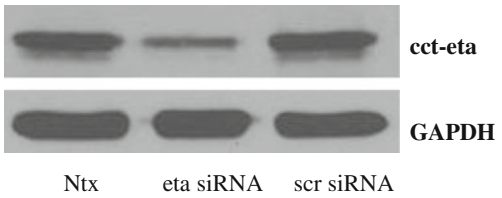

Fig. 4 siRNA versus CCT-eta inhibits CCT-eta protein expression in DC-derived fibroblasts. Western blot analysis of DC cells in culture shows that CCT-eta siRNA effectively inhibits CCT-eta protein expression (to 39±2 % of control Ntx; $p<0.007$), but a scrambled nonspecific control siRNA does not (97±1 % of control Ntx; $p<0.01$). GAPDH was used as a loading control. A representative immunoblot is shown. *Ntx* no treatment, *etasi* siRNA versus CCT-eta, *scrsiRNA* scrambled control siRNA

and Rayan (1995) found that DC cells that expressed a higher level of α-SMA were significantly more contractile in an FPCL assay than control CT cells, which on average expressed less α-SMA. Studies using culture force monitoring have shown that DC nodule-derived fibroblasts are more contractile than CT-derived cells (Bisson et al. 2004), and also more contractile than normal skin-derived fibroblasts (Verjee et al. 2010). FPCL assay has also shown that fibroblasts from active DC disease are more contractile than fibroblasts from patient-matched clinically normal palmar fascia (Howard et al. 2003), and that this inherent increased contractility does not entirely stem from endogenously secreted transforming growth factor-β (Tse et al. 2004). Our studies utilize fibroblasts from actual DC cord contractures, not nodules, and for the first time compare the contractility of both DC- and patient-matched PF-derived cells with CT-derived controls. The increased DC contractility we observe with both FPCL and CTFM assays is consistent with previously reported results indicating that DC fibroblasts are inherently more contractile than normal fibroblasts from CT patients. Interestingly, the FPCL assay also shows that PF-derived fibroblasts are intermediate in their contractile phenotype between CT and DC cells. While this distinction was not observed with the CTFM assay, this may be due to specific experimental limitations in the assay as employed

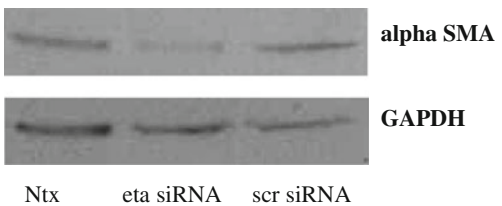

Fig. 5 siRNA versus CCT-eta inhibits α-SMA protein expression in DC-derived fibroblasts. Western blot analysis of DC cells in culture shows that CCT-eta siRNA effectively inhibits α-SMA protein expression (by 40.09±2.46 % compared to control Ntx; $p<0.001$), but a scrambled nonspecific control siRNA does not (98±0.17 % of control Ntx; $p<0.001$). GAPDH was used as a loading control. A representative immunoblot is shown. *Ntx* no treatment, *etasi* siRNA versus CCT-eta, *scrsiRNA* scrambled control siRNA

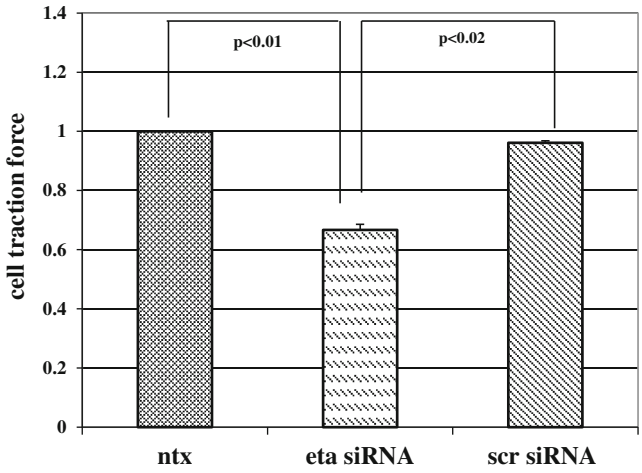

Fig. 6 siRNA versus CCT-eta inhibits cellular contractility in DC-derived fibroblasts. DC-derived fibroblasts were transfected with CCT-eta siRNA or scrambled control siRNA along with the marker plasmid PDSRedC1. Red fluorescent cells, signifying successful uptake of the transfecting constructs, were quantified for microdisplacement fields on the green fluorescent substrates. The assay was repeated twice, quantifying more than 15 cells for each condition in each experimental run. The values are normalized to baseline contractility (Ntx). Values are means±SEM of two independent experiments. Statistical analyses were performed using Student's *t* test, with *p* value <0.05 considered significant. CCT-eta siRNA transfected cells exhibited markedly decreased cellular traction force; this effect was not seen in cells transfected with a scrambled control siRNA. *Ntx* no treatment, *etasi* siRNA versus CCT-eta, *scrsiRNA* scrambled control siRNA

here, or it may be that the cumulative population effect measured in the FPCL assay is simply better at capturing this difference. The observation that PF cells have an intermediate contractile phenotype is of particular note since we have previously reported that PF cells also have an intermediate transcriptomic profile between CT and DC cells. Microarray examination of global gene expression in six DC and matched PF fibroblasts in tissue culture on collagen (as well as six CT-derived fibroblast controls) revealed that all PF cells closely resembled (but were not identical to) their DC counterparts, but both groups differed significantly from CT cells (Satish et al. 2012). It therefore appears that the increased contractility seen in PF cells is a phenotypic reflection of their status as latent disease cells, displaying characteristics that are intermediate between normal CT and active DC disease conditions.

This intermediate position of PF cells is further established by the observed patterns of expression of CCT-eta and α-SMA. Phenotypically normal CT cells, from which no contracture may be expected, express low levels of CCT-eta protein and low levels of α-SMA. PF cells, also from clinically normal tissue, express markedly increased CCT-eta, but only modest α-SMA. DC cells from active contracting disease, however, express both CCT-eta and α-SMA at increased levels. These striking observations indicate that

CCT-eta is an early marker for cells that are actively contributing to, or are capable of contributing to, contractures even if they have not become active and caused clinically evident disease. If recurrence or progression of DC contracture results from the activation or "transformation" of PF cells into DC cells, which seems a likely, albeit currently unproven, prospect, then CCT-eta may be an attractive target for molecular intervention with which to forestall disease.

For this to be the case, however, there would need to be a mechanistic connection between increased CCT-eta expression and the properties in DC fibroblasts that lead to fibrotic contraction of diseased tissues. We have previously reported that in fibrotic skin wound healing, CCT-eta expression increases in parallel with α-SMA expression (Satish et al. 2010b). A similar coincident rise in CCT-eta and α-SMA is seen in a model of corneal wound healing (Koulikovska et al. 2005). In contrast, CCT-eta is decreased in scarlessly healing fetal wounds, which also feature a paucity of myofibroblasts and little α-SMA expression (Cass et al. 1997; Satish et al. 2008, 2010b). CCT-eta and α-SMA expression, then, at least correlate with a fibrotic phenotype.

We have also reported that reduction of CCT-eta using a specific siRNA inhibits both fibroblast cell motility and contractile response to stimulatory agents (Satish et al. 2010a). siRNA-mediated inhibition of CCT-eta, but not its evolutionary homolog CCT-beta, simultaneously knocks down α-SMA protein expression, indicating that CCT-eta expression is permissive and necessary for α-SMA accumulation. Increased α-SMA expression is the hallmark of myofibroblastic conversion and correlates with increased contractility in Dupuytren's fibroblasts (Verjee et al. 2010; Tomasek and Rayan 1995). Increased α-SMA expression has also been positively correlated with severity of scar contraction in a model of hypertrophic burn scarring (Wang et al. 2011). Thus, interference with CCT-eta, and therefore α-SMA, would potentially prevent conversion of PF cells into functionally diseased DC cells, would potentially limit the deformational contractile ability of DC myo/fibroblasts, and ultimately may prevent manifestation of clinically evident fibrotic disease. Our results in this study suggest that at least part of the above is likely correct: siRNA-mediated inhibition of CCT-eta expression in the aberrantly hypercontractile DC-derived fibroblasts both significantly reduces α-SMA levels and inhibits their contractile function in the CTFM assay.

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