Amino Acids (2006) 30: 445–451
DOI 10.1007/s00726-005-0265-y

Amino Acids
Printed in Austria

Extracellular matrix containing mutated fibrillin-1 (*Fbn1*) down regulates *Col1a1*, *Col1a2*, *Col3a1*, *Col5a1*, and *Col5a2* mRNA levels in *Tsk/+* and *Tsk/Tsk* embryonic fibroblasts

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Received July 27, 2005
Accepted September 29, 2005
Published online April 4, 2006;

Summary. It is known that the extracellular matrix (ECM) is able to signal to cells and thereby direct or modulate the transcription of certain mRNAs. This signaling plays an important role in tumor invasion and metastasis, wound healing, remodeling of the ECM and cell differentiation. There are several mechanisms whereby the ECM signals cells to change their metabolism: (1) receptor molecules binding to specific domains in the ECM, (2) direct phagocytosis of the ECM molecules or domains into the cell, (3) structural changes of the ECM domains. We report the effect of an ECM containing either mutant or normal *Fbn1* on the transcription levels of several collagen mRNAs. *Tsk/Tsk*, *Tsk/+* and *+/+* mouse embryonic fibroblast cell lines were used. *Tsk/Tsk* cells produce only mutated fibrillin-1 which arises from mRNA containing an in-frame duplication of exons 17–40. To test the effect of the ECM containing mutant *Fbn1*, cells of the *Tsk/Tsk*, *Tsk/+* and the wild-type (+/+) genotype were each grown on an ECM produced by either *Tsk/Tsk*, *Tsk/+* cells or by wild-type cells (+/+). The embryonic cells were genotyped by Northern analyses for *Fbn1* and grown to confluence. The cultures were then harvested and the cells removed, leaving the matrix in the flasks. Matrices produced from *Tsk/Tsk*, *Tsk/+* and from *+/+* cells were reseeded with *Tsk/Tsk* cells, *Tsk/+* cells or *+/+* cells. The cells were plated at a confluent concentration and incubated on the matrices for 48 h, after which total RNA was harvested and cDNA generated. Real-time PCR using cDNA or Northern analyses using RNA were performed for *Fbn1* and Types I, III and V collagens. The PCR and Northern results were normalized using β-actin and GAPDH, respectively. The Northern analyses showed that the steady state levels of mRNA for *Col1a1* were depressed in both *Tsk/Tsk* and *+/+* cells when grown on the matrix produced by *Tsk/Tsk* cells. Real-time PCR was then performed with primers specific for *Col1a2*, *Col3a1*, *Col5a1* and *Col5a2*. The results showed that cells with the *Tsk/Tsk*, *Tsk/+*, and *+/+* genotype all had lower steady-state levels of the above 4 collagen mRNAs when grown on the matrix produced by homozygous *Tsk/Tsk* cells or the matrix produced by heterozygous *Tsk/+* cells compared with those grown on a matrix produced by *+/+* cells. We hypothesize that the mutated *Fbn1* molecules with many additional EGF-calcium binding regions and TGF-β binding domains may (1) change the homeostasis of the ECM by binding additional growth factors and/or (2) present a radically different ECM 3-dimensional architecture. Either or both of these changes could signal the cell to produce less collagen.

Keywords: Extracellular matrix – Fibrillin – Collagen – mRNA – Signaling – Scleroderma – Animal model – Mouse

Introduction

The idea that the matrix on which mammalian cells adhere is able to signal to the cell and thereby direct or modulate the transcription of certain mRNA species is not new. Such a mechanism has been reported to be a part of the method whereby malignant cells invade into normal tissue (Brader and Eccles, 2004; Mook et al., 2004) as well as part of wound healing, remodeling of the extracellular matrix (ECM) and cell differentiation (Rosenkranz, 2004; Klenkler and Sheardown, 2004; Eckes et al., 1999; Lambert et al., 2004). One can imagine several mechanisms whereby the matrix signals the cell to change its metabolism. First the ECM components must be recognized in some way by the cell. All cells are, of course, equipped with multiple cell surface receptors specifically designed to recognize ECM components both soluble and insoluble (Ffrench-Constant and Colognato, 2004). These receptor molecules bind to specific domains in the ECM and transmit a signal through the cell plasma membrane indicating that the receptor has found its ligand. The internal portion of the receptor then undergoes a change which allows the information to be transmitted to a soluble factor which can enter the nucleus and cause a change in the transcription of specific mRNAs. Alternatively, certain cells can directly phagocytize ECM molecules or domains into the cell after which the cell again transmits this discovery of a certain domain to the nucleus thus changing transcription (Jacob et al., 2002). A third mechanism by which transcription can be altered is through the actual structural design of the ECM domains (Lonai, 2003; Eckes et al., 2004). If, for example, the cell finds certain binding sites in the ECM to be in a

different position, its three-dimensional architecture (shape) would be altered. Such a mechanism would not require a receptor to directly transmit information by transduction or internalization but through a more indirect method whereby changing the overall cell shape would cause information to be transmitted to the nucleus. This pathway would still involve cell surface receptors.

In the present study we investigated what effect an ECM containing mutant or normal fibrillin-1 would have on the transcription of several collagen mRNAs. Embryonic cell lines from the tight skin mouse ($Tsk/+$) were used. The $Tsk/+$ mouse resulted from a spontaneous dominant mutation that occurred in the inbred B10.D2 (58N)/Sn strain. This mutation was identified at the Jackson Laboratories by Helen Bunker and reported in detail by Green et al. (1976). The most striking feature of heterozygous animals ($Tsk/+$) of this strain is the presence of thickened and tight skin which is firmly bound to the subcutaneous and deep muscular tissues. Tsk/Tsk homozygous embryos are not viable and degenerate in utero at 8–10 days of gestation. The heterozygous mice display cutaneous and visceral changes that closely resemble those present in patients with SSc. Furthermore, the biochemical and molecular abnormalities that have been demonstrated in these animals (Jimenez et al., 1984, 1988) mimic the connective tissue alterations characteristic of SSc, particularly those related to the fibrotic stage of the disease.

The $Tsk/+$ mouse has one copy of normal fibrillin and one mutant copy in which an approximate 3 kb in-frame insertion has been incorporated into the mRNA. Exons 17–40 are repeated (Siracusa et al., 1996). The resulting mutated protein contains approximately 1000 additional amino acids which consist of multiple additional EGF-calcium binding and TGF-β receptor repeats and one additional Fib motif and one RGD domain (integrin). It has been hypothesized that these additional domains would affect the homeostasis of the ECM because this mutated molecule would be able to bind additional growth factors. In vivo, it has been suggested that these additional domains could cause a higher influx of TGF-β and EFG-like molecules into the ECM. The additional availability of these growth factors would cause an increase in ECM synthesis. In fibroblast cultures where there are no other cell types or a large excess of extracellular growth factors, it was unclear what effect the additional mutant fibrillin domains might have on the synthesis of collagens which are secreted into the ECM. To test the effect of the mutant fibrillin, cells of the Tsk/Tsk and $Tsk/+$ genotype and of the wild type ($+/+$) were each grown on an ECM produced by Tsk/Tsk, $Tsk/+$ and wild-type cells ($+/+$).

Materials and methods

Tissue culture cells

Homozygous, Tsk/Tsk, heterozygous, $Tsk/+$, and normal, $+/+$, embryonic cell lines were prepared from crossing $Tsk/+ \times Tsk/+$ mice. Timed pregnancies were performed allowing for sacrifice of the pregnant female at 7.5 days in gestation, before the homozygous Tsk/Tsk mice degenerate and die at 8–10 days in utero. Embryos were dissected, freed of the surrounding tissue, minced and plated in 6 well plates in DMEM with 20% FBS. Cells from 30 different embryos were grown until multiple T75 flasks were obtained from each dissected embryo. Northern blot analysis was performed on two flasks (see Fig. 2) for each embryo with a probe for *Fbn1* in order to genotype the cell line. The remaining flasks were used to supply matrix and cells.

Preparation of cell matrix free of original producing cells

Confluent T75 flasks were plated into 100 mm dishes, cultured until confluent and treated with 1 × lysis buffer (product nr. 1897675 Roche, Indianopolis, IN). The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere for 4 h. Dishes were viewed under a microscope to assure that cells were destroyed and the matrix was still intact. Following treatment, 4 ml of media was added to the dishes and incubated for 1 h at the previous conditions. The media was changed with fresh media and the matrices were incubated overnight, after which the flasks were again examined microscopically to insure that the matrix was intact and that no cells were alive. To insure that the cells had been completely lysed by the above treatment, 4 flasks of Tsk/Tsk, $Tsk/+$, and $+/+$ cells (12 flasks in total) were treated with the above lysis buffer, stained with Trypan blue exclusion dye and viewed under a phase inverted microscope. The results (Fig. 1) show that no live cells were observed in any of the flasks. In addition, the number of dead cells per microscopic field did not vary by more than 10% between any of the three cell types.

Culturing of Tsk/Tsk, Tsk/+, and +/+ cells on Tsk/Tsk and +/+ matrices

Confluent Tsk/Tsk, $Tsk/+$, and $+/+$ cells were washed 2 times with 1 × Dulbecco's phosphate-buffered saline. They were treated with 0.25% Trypsin-EDTA to break cellular adhesion molecules so cells can be freed from their growing surface. The cells were removed from the flasks and evenly distributed onto the appropriate matrices at a confluent concentration and incubated for 24 h. The cells were then treated with ascorbic acid (50 μg/ml) and harvested after another 24 h.

Northern blot analyses

RNeasy Mini Kit (Qiagen) was used to isolate total RNA. The purity of these extractions was checked with the Gene Quant II RNA/DNA calculator (Pharmacia Biotech) at 260 nm. Aliquots (15 μg/per well) were precipitated using the speed vacuum and brought up to volume with double distilled water. The samples were electrophoresed on 1% agarose containing formaldehyde and MOPS. The RNA was transferred to Hybond N+ filters (Amersham Biosciences) and hybridized to ^{32}P radiolabelled fibrillin-1, *Col1a1*, and GAPDH.

Quantification of Northern blots by phosphoimaging and image-quant software

The filters were analyzed using densitometry equipment and software (Image Quant version 5.1; Amersham Biosciences). Equivalent loading and transfer of the RNA was verified by quantitative image analysis of ethidium bromide stained ribosomal RNA bands (data not shown). The signals of GAPDH were used to normalize all data because it has been shown by us (Christner et al., 1995, 1998) and others (Philips et al., 1995;

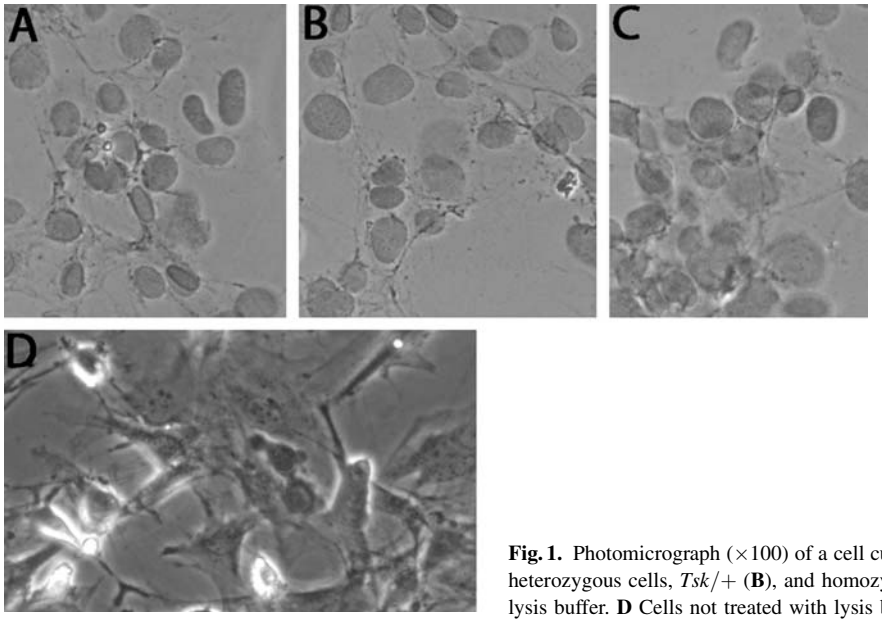

Fig. 1. Photomicrograph (×100) of a cell culture flask containing normal cells, +/+ (**A**), heterozygous cells, *Tsk*/+ (**B**), and homozygous cells, *Tsk*/*Tsk* (**C**), after treatment with lysis buffer. **D** Cells not treated with lysis buffer

Rosenbloom et al., 2000) that GAPDH is a reliable house keeping gene for measuring collagen mRNA levels by Northern blots as long as equivalent loading and transfer of RNA were verified by quantitative image analysis of ethidium bromide stained ribosomal RNA bands.

Real-time PCR

RNA was prepared as above and then used as template to produce cDNA with SuperScript III First Strand kit (Invitrogen). Probes were designed using Primer Express 2.0 v software (Applied Bioscience). Specificity was checked using the NCBI BLAST website. The PCR was carried out in a MyiQ real-time PCR machine (Bio-Rad Laboratories) with cyber green (Cyber Green PCR Master Mix, Applied Biosystems) as the reporter. MyiQ Optical System software 1.0 v was used (Bio-Rad Laboratories) to analyze the data. All results were normalized to the amount of β-actin in the sample such that β-actin was equal to 100. β-Actin was used as the housekeeping gene of choice to normalize this data for the following reasons: (1) it has been validated in RT-PCR for RNA extracted from two different populations of human fibroblasts (Redlich, 2004); (2) in Northern blot analyses, β-actin has been shown to be an adequate housekeeping gene for measuring changes in collagen mRNAs isolated from human (Diamond et al., 2003), chicken (Quinones et al., 1986), and *Tsk* mouse fibroblasts (Jimenez et al., 1986); and (3) microarray experiments which we performed comparing the expression of β-actin mRNA isolated from +/+ and *Tsk*/*Tsk* fibroblast lines showed that the β-actin mRNA expression in *Tsk*/*Tsk* fibroblasts was 15% lower than in controls (P. J. Christner, unpubl. obs., 2004).

Statistical analysis

P-values for a confidence level of 95% were obtained using the Chi-square method with the Yates correction (Software program Instat, version 3.05 by Graph Pad, San Diego, CA).

Results

Timed pregnancies were performed to obtain 30 embryos at 7.5 days of gestation. Seven and a half days of gestation was chosen because the *Tsk*/*Tsk* homozygous embryos degenerate and die at 8 days of gestation. Embryos were then minced and plated to obtain embryonic fibroblasts of the *Tsk*/*Tsk*, *Tsk*/+, and +/+ genotypes. The fibroblasts from each embryo were genotyped by extracting the total RNA and performing a Northern blot analysis for fibrillin-1. The results for 11 embryonic fibroblast cultures are shown in Fig. 2. It can be seen that lanes 5 and 8 show a mutant fibrillin transcript of 14,000, that lanes 1, 4, 7 and 10 show two transcripts of 14,000 and 11,000, and lanes 2, 3, 6, 9, and 11 show only the normal transcript of 11,000. It should be noted that harvesting embryos at 7.5 days did not yield the expected 25% homozygous, 50% heterozygous, and 25% normal for a $Tsk/+ \times Tsk/+$ cross. Of the 30 embryos obtained, only 3 were *Tsk*/*Tsk*.

After the embryonic cells had been genotyped, they were grown to confluence and further incubated for 24 to 48 h. The cultures were then harvested and the cells killed

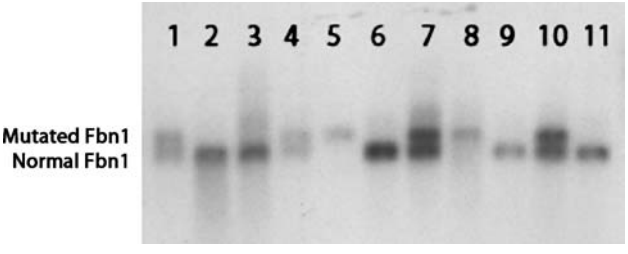

Fig. 2. Northern blot of RNA derived from 11 different embryonic cell lines. Each cell line was derived from a pregnant *Tsk*/+ female (crossed to a *Tsk*/+ male) at 7.5 days of gestation. The blot was probed with ^{32}P-labeled murine *Fbn1*. Lanes 1, 4, 7, and 10 show two transcripts with molecular weights of 14,000 and 11,000; lanes 2, 3, 6, 9, and 11 show only the normal transcript of 11,000; lanes 5 and 8 show a mutant fibrillin transcript of 14,000

as described in the Materials and methods section, leaving the matrix still in the flasks. Matrices produced from *Tsk/Tsk*, *Tsk/+*, and +/+ cells were obtained. Three cell lines of each genotype were used. Each of these matrices was then reseeded with confluent *Tsk/Tsk* cells, *Tsk/+* cells, or +/+ cells. Control matrices were also incubated without cells. The cells were allowed to incubate on the matrices for 48 h after which the RNA was harvested. No measurable RNA was recovered from the control flasks containing only matrix. Northern blot analyses were performed for type 1 collagen (*Col1a1*), and GAPDH using only *Tsk/Tsk* cells, or +/+ cells. The results of these Northern blots are shown in Fig. 3. Inspection of Fig. 3 shows that the steady-state levels of mRNA for *Col1a1* are reduced when the homozygous embryonic cells were plated on the matrix derived from the homozygous cell lines (lanes 10–12) compared to when they were plated on normal cells (lanes 1–3). Similarly, the steady-state levels of mRNA for *Col1a1* are also reduced when the normal embryonic cells were plated on the matrix derived from the homozygous cell lines (Fig. 3, lanes 7–9) compared to when they were plated on a matrix derived from normal cells (Fig. 3, lanes 4–6). The results in Fig. 4 show the quantitative difference in the *Col1a1* values after they were normalized to the GAPDH values for each lane. The results in Fig. 4 were obtained by scanning the Northern blot with a densitometer. It can be seen that when *Tsk/Tsk* mutant cells were grown on the matrix containing only mutated fibrillin-1, the steady-state level of *Col1a1* mRNA was approximately 40% of the level measured when these cells were grown on a matrix containing normal fibrillin-1. Similarly when normal (+/+) cells were grown on the matrix containing only mutated fibrillin-1, the steady-state level of *Col1a1* mRNA was approximately 80% of the level measured when these cells were grown on a matrix containing normal fibrillin-1.

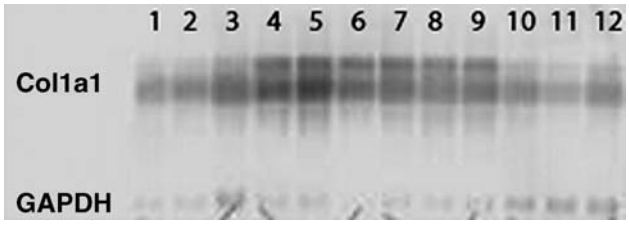

Fig. 3. Northern Blot of RNA obtained from *Tsk* embryonic fibroblasts grown on a matrix containing mutant or normal *Fbn1*. The blot was hybridized with a rat probe for *Col1a1* and a human probe for GAPDH. Lanes 1–3 show the results of RNA extracted from *Tsk/Tsk* cells homozygous for mutant *Fbn1* grown on a matrix produced by normal (+/+) embryonic fibroblasts; lanes 4–6 show the results of RNA from normal (+/+) cells grown on a matrix produced by normal (+/+) embryonic fibroblasts; lanes 7–9 show the results of RNA from normal (+/+) cells grown on a matrix produced by *Tsk/Tsk* embryonic fibroblasts homozygous for mutant *Fbn1*; lanes 10–12 show the results of RNA from *Tsk/Tsk* cells homozygous for mutant *Fbn1* grown on a matrix produced by *Tsk/Tsk* embryonic fibroblasts

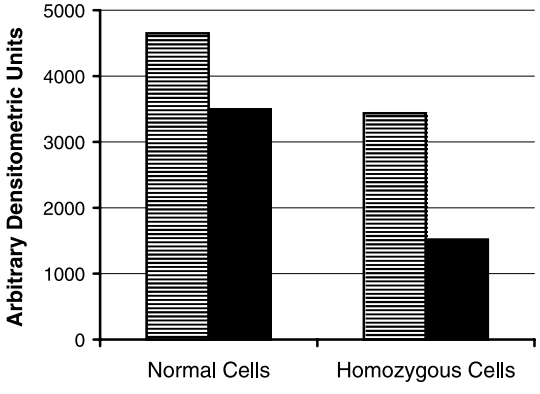

Fig. 4. Quantification of a Northern blot probed with *Col1a1*, scanned with a densitometer and normalized to GAPDH. The steady-state levels of *Col1a1* mRNA are shown for *Tsk/Tsk* cells (homozygous for mutated *Fbn1*) and +/+ cells (normal) grown on either a matrix produced by *Tsk/Tsk* (black bars) cells (homozygous for mutated *Fbn1*) or by +/+ cells (striped bars) (normal)

By further experiments using real-time PCR, the message levels for *Col1a2*, *Col3a1*, *Col5a1*, and *Col5a2* were quantified and relative expression of each message was determined after normalization to the expression of β-actin. The results in Fig. 5 show that when the cells which were

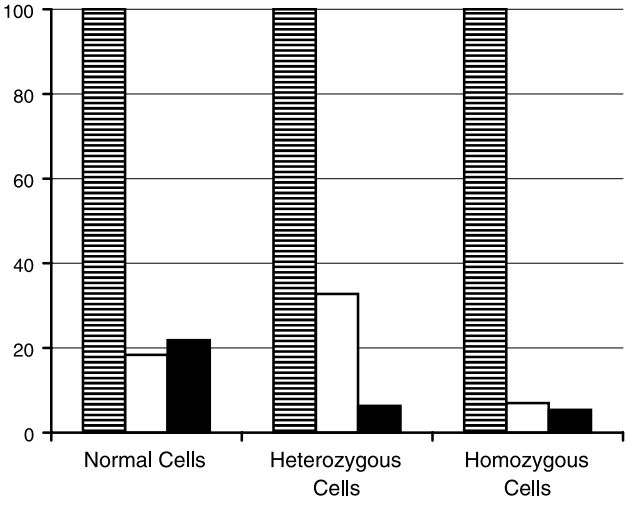

Fig. 5. Results of a real-time PCR which amplified *Col1a2* cDNA produced from mRNA extracted from homozygous (*Tsk/Tsk*) cells, heterozygous (*Tsk/+*) cells, and normal (+/+) cells. The cells were grown on a matrix produced by normal (+/+) (striped bars), heterozygous (*Tsk/+*) (white bars), or homozygous (*Tsk/Tsk*) cells (black bars). Relative expression was normalized to β-actin and is expressed as percentage of the expression of *Col1a2* when grown on normal (+/+) matrix

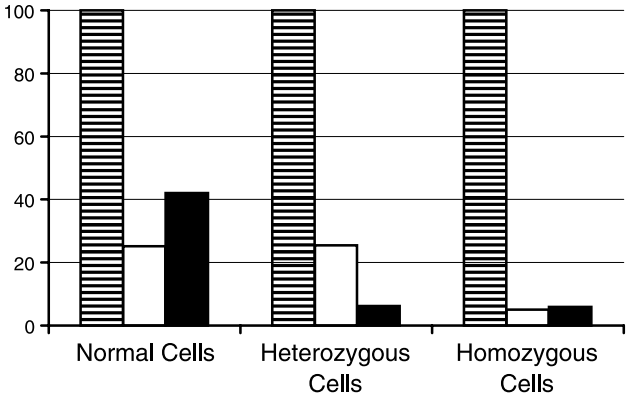

Fig. 6. Results of a real-time PCR which amplified *Col3a1* cDNA produced from mRNA extracted from homozygous (*Tsk*/*Tsk*) heterozygous (*Tsk*/+) and normal (+/+) cells. The cells were grown on a matrix produced by normal (+/+) (striped bars), heterozygous (*Tsk*/+) (white bars), or homozygous (*Tsk*/*Tsk*) cells (black bars). Relative expression was normalized to β-actin and is expressed as percentage of the expression of *Col3a1* when grown on normal (+/+) matrix

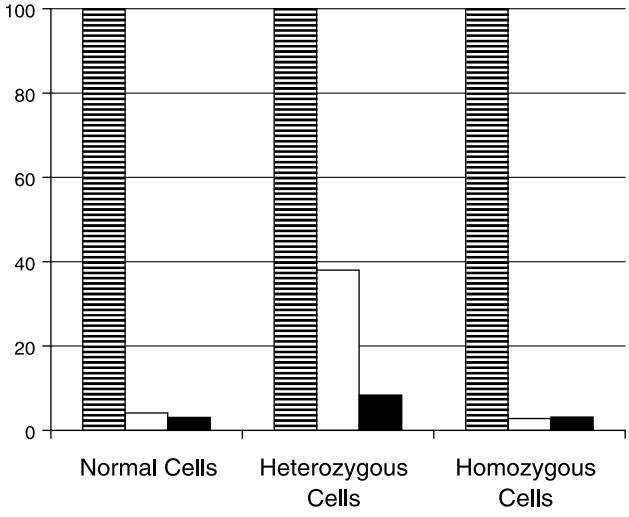

Fig. 7. Results of a real-time PCR which amplified *Col5a1* cDNA produced from mRNA extracted from homozygous (*Tsk*/*Tsk*) cells, heterozygous (*Tsk*/+) cells and normal (+/+) cells. The cells were grown on a matrix produced by normal (+/+) (striped bars), heterozygous (*Tsk*/+) (white bars), or homozygous (*Tsk*/*Tsk*) cells (black bars). Relative expression was normalized to β-actin and is expressed as percentage of the expression of *Col5a1* when grown on normal (+/+) matrix

homozygous (*Tsk*/*Tsk*), heterozygous (*Tsk*/+), or normal (+/+) for *Fbn1* were cultured on a matrix containing mutated fibrillin-1, the relative expression for *Col1a2* was dramatically reduced compared to the relative expression of *Col1a2* when these cells were cultured on normal matrix. These differences were significant to a $p < 0.01$. In addition, smaller differences in the expression of *Col1a2* were observed when the cells were grown on heterozygous matrices or homozygous matrices. However, these differences were not significant. In Fig. 6, the results for the expression of *Col3a1* message levels show that when cells derived from *Tsk*/*Tsk*, *Tsk*/+, or +/+ embryos were cultured on a matrix containing mutated fibrillin-1, *Col3a1* mRNA levels were also markedly reduced compared to when they were cultured on a normal matrix ($p < 0.01$). The difference in expression of the *Col3a1* message between cells grown on heterozygous and homozygous matrix was not significant. The results for the expression of the *Col5a1* message levels are shown in Fig. 7. Similarly, the expression of this mRNA species was significantly lower when any of the three types of fibroblasts were cultured on matrices derived from heterozygous or homozygous embryos compared to when they were cultured on matrices derived from normal embryos. This difference was significant at $p < 0.01$. The smaller difference in expression of *Col5a1* mRNA observed between cells grown on heterozygous and cells grown on homozygous matrices was not significant. Message levels for *Col5a2* showed that the expression of this mRNA species was similarly depressed when the cells were cultured on matrices containing mutated fibrillin-1 compared

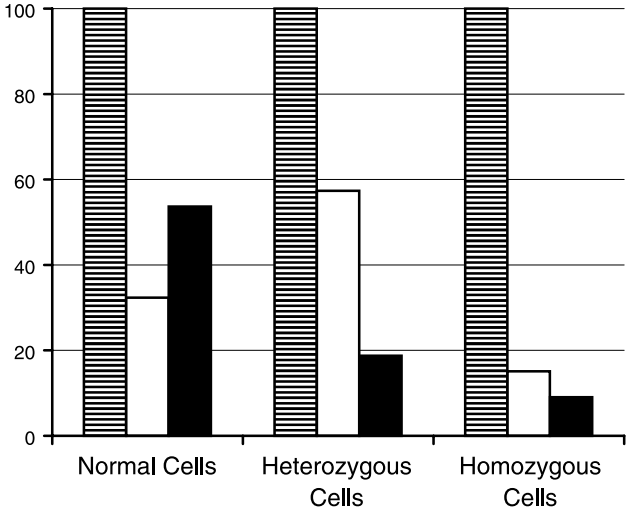

Fig. 8. Results of a real-time PCR which amplified *Col5a2* cDNA produced from mRNA extracted from homozygous (*Tsk*/*Tsk*) cells, heterozygous (*Tsk*/+), cells and normal (+/+) cells. The cells were grown on a matrix produced by normal (+/+) (striped bars), heterozygous (*Tsk*/+) (white bars), or homozygous (*Tsk*/*Tsk*) cells (black bars). Relative expression was normalized to β-actin and is expressed as percentage of the expression of *Col5a2* when grown on normal (+/+) matrix

to when they were cultured on matrices containing normal fibrillin-1. These differences were significant at a $p < 0.01$, except that difference in expression observed for this mRNA species in heterozygous (*Tsk*/+) derived cells cultured on heterozygous matrices was significant at $p < 0.05$.

Discussion

Together, the above results show that the mutated fibrillin-1 protein containing an in-frame duplication of exons 17–40 (Siracusa et al., 1996) which is produced by *Tsk/Tsk* and *Tsk/+* embryonic fibroblasts has the ability to down regulate the mRNA levels for types I, III, and V collagens in mouse embryonic fibroblasts, whether or not these cells themselves are homozygous, heterozygous, or normal for the presence of the mutant fibrillin-1 gene. The highest levels of depression for *Col1a2*, *Col3a1*, *Col5a1*, and *Col5a2* were observed in *Tsk/Tsk* embryonic fibroblasts. *Col1a1* for which only data on *Tsk/Tsk* and +/+ cells were measured also showed that the depression was higher in *Tsk/Tsk* embryonic fibroblasts. The intriguing question is how the mutant fibrillin-1 in the ECM causes the cell to down regulate these mRNAs. Several investigators have reported that the ECM produced by *Tsk/+* mice is abnormal. Kielty et al. (1998) have reported that the *Fbn1* polymerizes and becomes incorporated into a discrete population of beaded microfibrils with altered molecular organization. They found altered microfibril abundance, changed accessibility for *Fbn1* epitopes and changes in the ECM. Electron microscopic studies found blurred appearance with no obvious periodicity. Ultrastructural examination showed that the classic beaded microfibrils existed as two distinct populations. They postulated that the aberrant population of microfibrils would have altered surface interactive properties which resulted from misalignment, misfolding and/or aberrant cross-linking and these changes would alter growth factor binding potential. All these changes could be expected to affect the message levels for proteins which are secreted to become part of the ECM. Interestingly, these results are different from those reported by Jimenez et al. (1986) for dermal fibroblasts: that the message levels for type I and III collagen are up-regulated in those cells. Lemaire et al. (2004a), who also studied mouse dermal fibroblasts, reported no increase in either *Col1a1* message levels or collagen secretion in the cells producing mutated *Fbn1* compared to controls. However, there are critical differences between these earlier studies and what is reported here. Our studies are with embryonic fibroblasts derived very early in gestation and compare the influence of an artificial matrix, which has been produced by embryonic cell lines and contains only mutated fibrillin-1, a mix of 50% normal and 50% mutant fibrillin-1, or all normal fibrillin-1. Our results suggest that 50% mutant fibrillin-1 is enough to have a large effect on the message levels of collagens I, II, and V.

Both Lemaire et al. (2004a) and Kielty et al. (1998) concluded that mutated *Fbn1* is associated with altered composition of the ECM. Lemaire et al. (2004a) postulated that the altered fibrillin-1 incorporated into the ECM would lead to abnormal interaction between the mutated protein and other ECM molecules, which could lead to increased collagen deposition. It is possible that changing the mixture from part normal to part mutated fibrillin-1 or all mutated fibrillin-1 could cause a different response in these cells. The down regulation of the collagen mRNAs was greatest in cells grown on homozygous matrix and the fact that the homozygous mice (*Tsk/Tsk*) are not viable would suggest that the effect of having only mutant fibrillin-1 in the ECM would be extreme. However, because we did not find a significant difference in the down regulation of the collagen mRNAs when cells were grown on heterozygous compared to homozygous matrices, it is possible the total lack of normal fibrillin-1 may lead to other alterations in the growth and development of the mouse embryo which makes them nonviable.

Unfortunately, the two studies of Lemaire et al. (2004a) and Kielty et al. (1998) do not shed light on how the ECM containing mutated fibrillin-1 would send back a signal to the cell to decrease (or increase) collagen message levels. In more recent work Lemaire et al. (2004b) do report an altered ECM in *Tsk/+* containing increased amounts of fibrillin-1, fibulin-2, and MAGP-2 and they postulate that this type of change in the ECM might transmit a signal to fibroblasts through integrins, because these proteins, which are increased in the *Tsk/+* ECM, bind to integrins and contain RGD domains. Finally, Eckes and Krieg (2004) have shown that mechanical tension can be regarded as an additional important regulatory parameter through which the ECM can signal to the cell. Therefore, cells grown on an aberrant ECM containing mutated fibrillin-1 which has clearly been shown to have a vastly different architecture (Kielty et al., 1998) could transmit a signal through its different architecture. Having only mutant fibrillin-1 with no normal copies of this protein in the matrix could be expected to cause greater changes in the architecture of the matrix than when there is a mixture of mutant and normal fibrillin-1 present.

Our work clearly shows that in tissue culture the ECM produced by heterozygous and homozygous *Tsk/Tsk* embryonic fibroblasts causes a significant decrease in collagen message levels compared to an ECM produced by normal, +/+, embryonic fibroblasts. Whether this phenomenon is through integrin signaling because of an altered ECM architecture of these receptors or some other mechanism is not yet known. However, this system would

be ideal to study how the matrix can signal to the cell to down regulate the collagen mRNAs.

Acknowledgment

This study was supported by NIH grants AR49541, AR42666 and AR07583.

References

Brader S, Eccles SA (2004) Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis. Tumori 90: 2–8

Christner PJ, Peters J, Hawkins D, Siracusa LD, Jimenez SA (1995) The Tight Skin (Tsk 2) mouse: an animal model of scleroderma displaying cutaneous fibrosis and mononuclear cell infiltration. Arthritis Rheum 38: 1791–1798

Christner PJ, Hitraya EG, Peters J, McGrath R, Jimenez SA (1998) Transcriptional activation of the α1(I) procollagen gene and upregulation of α1(I) and α1(III) collagen mRNA's in Tight Skin 2 (Tsk2) dermal fibroblasts. Arthritis Rheum 41: 2132–2142

Diamond MP, El-Hammady E, Wang R, Saed G (2003) Regulation of transforming growth factor-beta, type III collagen, and fibronectin by dichloroacetic acid in human fibroblasts from normal peritoneum and adhesions. Fertil Steril 7: 1161–1167

Eckes B, Kessler D, Aumailley M, Krieg T (1999) Interactions of fibroblasts with the extracellular matrix: implications for the understanding of fibrosis. Springer Semin Immunopathol 21: 415–429

Eckes B, Krieg T (2004) Regulation of connective tissue homeostasis in the skin by mechanical forces. Clin Exp Rheumatol 22 (3 Suppl 33): S73–76

Ffrench-Constant C, Colognato H (2004) Integrins: versatile integrators of extracellular signals. Trends Cell Biol 14: 678–686

Green MC, Sweet HO, Bunker LE (1976) Tight-skin, a new mutation of the mouse causing excessive growth of connective tissue and skeleton. Am J Pathol 82: 493

Jacob SS, Shastry P, Sudhakaran PR (2002) Monocyte-macrophage differentiation in vitro: modulation by extracellular matrix protein substratum. Mol Cell Biochem 233: 9–17

Jimenez SA, Millan A, Bashey RI (1984) Scleroderma-like alterations in collagen metabolism occurring in the *TSK* (tight skin) mouse. Arthritis Rheum 27: 180

Jimenez SA, Bashey RI, Williams CJ, Millan AH (1988) The tight skin (*TSK*) mouse as an experimental model of scleroderma. In: Greenwald RA, Diamond HS (eds) CRC handbook of animal models for rheumatic diseases. CRC Press, Boca Raton, pp 169–192

Kielty CM, Raghunath M, Siracusa LD, Sherratt MJ, Peters R, Shuttleworth CA, Jimenez SA (1998) The tight skin mouse: demonstration of mutant fibrillin-1 production and assembly into abnormal microfibrils. J Cell Biol 140: 1159–1166

Klenkler B, Sheardown H (2004) Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Exp Eye Res 79: 677–688

Lambert E, Dasse E, Haye B, Petitfrere E (2004) TIMPs as multifacial proteins. Crit Rev Oncol Hematol 49: 187–198

Lemaire R, Farina G, Kissin E, Shipley JM, Bona C, Korn JH, Lafyatis R (2004a) Mutant fibrillin 1 from tight skin mice increases extracellular matrix incorporation of microfibril-associated glycoprotein 2 and type I collagen. Arthritis Rheum 50: 915–926

Lemaire R, Korn JH, Schiemann WP, Lafyatis R (2004b) Fibulin-2 and fibulin-5 alterations in tsk mice associated with disorganized hypodermal elastic fibers and skin tethering. J Invest Dermatol 123: 1063–1069

Lonai P (2003) Epithelial mesenchymal interactions, the ECM and limb development. Anatomy 202: 43–50

Mook OR (2004) The role of gelatinases in colorectal cancer progression and metastasis. Biochim Biophys Acta 1705: 69–89

Philips N, Bashey RI, Jimenez SA (1995) Increased alpha 1(I) procollagen gene expression in tight skin (TSK) mice myocardial fibroblasts is due to a reduced interaction of a negative regulatory sequence with AP-1 transcription factor. J Biol Chem 270: 9313–9321

Quinones SR, Neblock DS, Berg RA (1986) Regulation of collagen production and collagen mRNA amounts in fibroblasts in response to culture conditions. Biochem J 239: 179–183

Redlich M, Roos H, Reichenberg E, Zaks B, Grosskop A, Bar Kana I, Pitaru S, Palmon A (2004) The effect of centrifugal force on mRNA levels of collagenase, collagen type-I, tissue inhibitors of metalloproteinases and beta-actin in cultured human periodontal ligament fibroblasts. J Periodontal Res 39: 27–32

Rosenbloom J, Saitta B, Gaidarova S, Sandorfi N, Rosenbloom JC, Abrams WR, Hamilton AD, Sebti SM, Kucich U, Jimenez SA (2000) Inhibition of type I collagen gene expression in normal and systemic sclerosis fibroblasts by a specific inhibitor of geranylgeranyl transferase I. Arthritis Rheum 43: 1624–1632

Rosenkranz S (2004) TGF-beta1 and angiotensin networking in cardiac remodeling. Cardiovasc Res 63: 423–432

Siracusa LD, McGrath R, Ma Q, Moskow JJ, Manne J, Christner PJ, Buchberg AM, Jimenez SA (1996) A tandem duplication within the fibrillin 1 gene is associated with the mouse tight skin mutation. Genome Res 6: 300–313

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