The effects of the mechanical stretch on human dermal fibroblasts

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The effects of the mechanical stretch on human dermal fibroblasts

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Sungsik Shin

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This certifies that the Master's Thesis
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Abstract

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Mechanical force plays a significant role in scar development which is supported by the fact that many high stretching tension areas tend to be keloids or hypertrophic scars. Furthermore, mechanical tension activates resting fibroblasts into activated phenotype which have the features of proliferation and matrix synthesis, and mechanical force has been shown to stimulate the transformation of dermal fibroblast into myofibroblasts, which is one of the important key mediators of fibrosis. Therefore, we aimed to examine the effects of mechanical stretch on proteins expressed in human dermal fibroblasts (HDFs) using

proteomics to find out the differences in expression of the proteins according to the presence or absence of stretching.

To generate the mechanical stretch, we made a mechanical cell stimulation machine which has a vacuum induced programmable biostretching system. The device applied multidirectional stretch and it was possible to control real-time manipulation to the attached cells. The mechanical stretch was applied to HDFs 2 hours per day for 3 days. Proteomics analysis was performed to compare the protein expressions between stretched HDFs and non-stretched HDFs.

After mechanical stretch, proliferation of HDFs was significantly increased than non-stretched HDFs. Proteomic analysis using TMT-labeling was performed to find altered protein expression after stretching and a total of 27 proteins showed significantly different expression between study and control groups. Among these proteins, 16 proteins were upregulated and 11 proteins were downregulated.

In the future, validating study will be needed for functional verification of each protein and evaluating the association with the pathway of keloid development and a better understanding of the effects of mechanical stretch on human fibroblasts will help the development of

novel technologies that can prevent or reduce pathological scar formation in the future.

Key words : human dermal fibroblasts (HDFs), mechanical stretch, keloid, scar formation, proteomics

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I. INTRODUCTION

Keloids and hypertrophic scars are skin abnormalities that are characterized by excessive deposition of collagen in the dermis and subcutaneous tissues resulting from unbalanced tissue homeostasis during normal wound healing, which can sometimes cause cosmetic and functional impairment such as disfigurement, contractures, pruritus and pain.[1]

Although there have been proposed a variety of mechanisms to explain keloid development, the pathogenesis of keloid remains still unclear. It is considered to be associated with chronic inflammation by various inflammatory cells

such as mast cell, macrophage and lymphocytes. Mechanical stretching is also considered to play a critical role in the development of keloid and ischemia of the surrounding blood vessels due to the proliferation of myofibroblast and endothelial cell can affect the development of keloid.[2]

Among them, mechanical theory is the theory that the development and progression of keloid is closely associated with the mechanical stretching. This theory is supported by the fact that many high stretching tension areas tend to be keloids or hypertrophic scars such as anterior chest and shoulder. In contrast, the areas with low tension and less movement such as scalp and tibia rarely occur keloid well. Furthermore, characteristic dumbbell shape of keloids is closely related with the direction of the skin tension.[3-5]

In previous research, mechanical tension activated resting fibroblasts into the activated phenotype which had the features of proliferation and matrix synthesis. In contrast, resting fibroblasts transformed in to the inflammatory phenotype which had the features of inhibition of proliferation and matrix degradation when the mechanical tension was not applied.[6,7]

Furthermore, mechanical force has been shown to stimulate the transformation of dermal fibroblast into myofibroblasts,[7] which is one of the important key mediators of fibrosis.[8]

In addition to this, some authors suggested that keloid fibroblasts are different from the normal fibroblasts and they have a critical role in development of

keloids, which synthesize excessive extracellular matrix (ECM) components such as collagen, fibronectin, elastin, and proteoglycans. The density of fibroblasts is increased in keloids compared to normal tissues, and the fibroblasts in keloids show resistance to apoptotic signal compared to normal fibroblasts.[1,9,10] And expression of matrix metalloproteinases (MMPs) and MMP tissue inhibitors are also skewed in keloids.[11,12]

Therefore, we aimed to examine the effects of mechanical stretch on proteins expressed in human dermal fibroblasts (HDFs) using proteomics to find out the differences in expression of the proteins according to the presence or absence of stretching.

II. MATERIALS AND METHODS

1. Cell cultures

Human dermal fibroblasts (HDFs) were prepared and treated in a tissue incubator with 0.1% gelatin and culture media comprising Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/strepromycin (Gibco). The cells were expanded at 37 ℃ with 5% CO2 and 90% humidity until they reached 80-90% confluence, after which they were passaged.

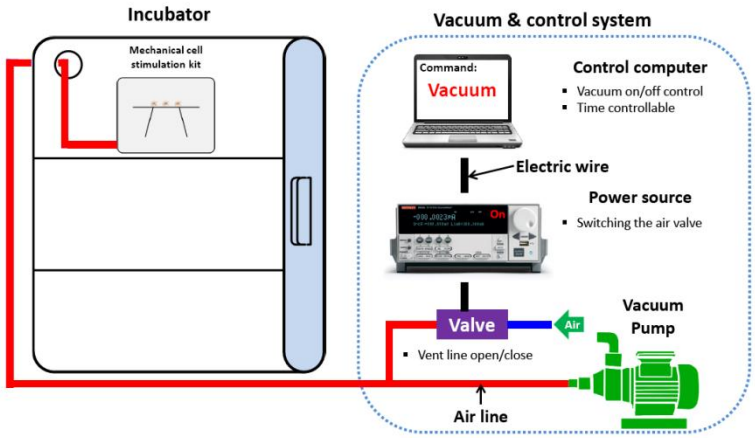
2. Application of mechanical stretch

To generate the mechanical stretch, we made special mechanical cell stimulation kit which is a vacuum induced programmable biostretching system called biostretching machine (BSM). The device consists of 4 x 6 array cells and is tightly connected to the cell media well via functionalized Polydimethylsiloxane (PDMS) membrane. The PDMS chamber measured 10 x 10 x 18 mm in its dimensions. Fibroblasts were seeded into a PDMS chamber at a density of 3 x 10^4 cells/chamber in 0.5 ml of culture medium. The device could apply multidirectional stretch unlike the previous device

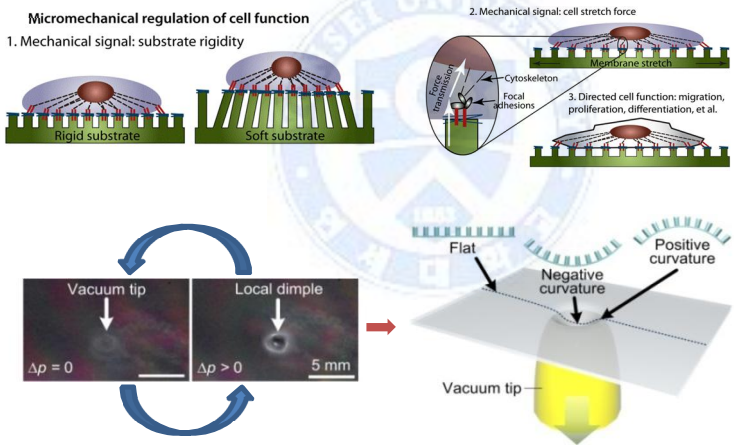
could only apply uniaxial or biaxial stretch. The multidirectional sinusoidal stretch (60cycles min^{-1}) was applied at 37 ℃, 5% CO2. And it was possible to control real-time manipulation to the attached cells (Figure 1).[13]

The cell cultures were done for one week in total. Stretching of more than 1 hour per day or stretching of more than 3 days showed a tendency to decrease cell viability. Thus the sinusoidal mechanical stretch was applied to HDFs 1 hour per day for only initial 3 days of culture period under the pressure of 5 kPa. The control group was incubated as described above without stretching.

A)


Incubator
Mechanical cell stimulation kit
Vacuum & control system
Command:
Vacuum
Control computer
Vacuum on/off control
Time controllable
Electric wire
Power source
Switching the air valve
Valve
Air
Vacuum Pump
Vent line open/close
Air line

B)


Micromechanical regulation of cell function
1. Mechanical signal: substrate rigidity
Rigid substrate
Soft substrate
2. Mechanical signal: cell stretch force
Membrane stretch
Force transmission
Cytoskeleton
Focal adhesions
3. Directed cell function: migration, proliferation, differentiation, et al.
Vacuum tip
Local dimple
Δp = 0
Δp > 0
5 mm
Flat
Positive curvature
Negative curvature
Vacuum tip

C)

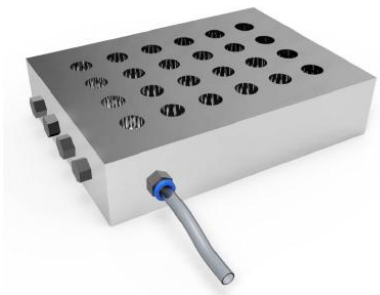


Figure 1. A) The schematic figure showing the configuration of the device. The mechanical cell stimulator is connected to the vacuum induced programmable control system. B) The figure showing the aspect of stretching within the mechanical cell stimulation kit. The device is using functionalized PDMS membrane, thus the multidirectional mechanical force and real-time manipulation of mechanical stress can be applied to the attached cells. C) The figure of the mechanical cell stimulation kit.

3. Proteome sample preparation

Mixtures of protein from each cell population were reduced with 500 mM Tris (2-carboxyethyl) phosphine at room temperature for 60 min and then alkylated with 500 mM IAA at room temperature in the dark for 60 min. The protein samples were then desalted using membrane filters (10-kDa molecular weight cutoff) and dissolved in 200 mM triethylammonium bicarbonate (TEAB) buffer to a final concentration of 1 μg/μl. Protein concentration was measured using a bicinchoninic acid assay (Thermo Scientific), following the manufacturer's protocol. Sequencing-grade trypsin (Promega, Madison, WI, USA) was added at an enzyme to protein ratio of 1:20 (wt/wt) in TEAB buffer and incubated overnight at 37°C. Samples were individually labeled using TMT-126, -128 and -130 (stretched group), and TMT-127, -129 and -131 (nonstretched group), following the manufacturer's protocol (Thermo Scientific). Aqueous hydroxylamine solution (5% w/v) was added to quench the reaction. The six samples were then combined, speed-vacuum dried, and re-dissolved in 50 l of water containing 0.1% formic acid for 2D-LC-MS/MS analysis.

4. 2D-LC-MS/MS

The TMT-labeled samples were analyzed using a 2D-LC-MS/MS system consisting of a nanoACQUITY UltraPerformance LC System (Waters,

Milford, MA, USA) and an LTQ Orbitrap Elite mass spectrometer (Thermo Scientific) equipped with a nano-electrospray source. Briefly, a strong cation-exchange column (5 m, 3 cm) was placed just before the C18 trap column (180- m i.d., 20-mm length, and 5- m particle size; Waters). A 5-µl aliquot of peptide sample was loaded for each 2D-LC-MS/MS run. Peptides were displaced from the strong cation-exchange column to the C18 column using a salt gradient that was introduced through an autosampler loop. The peptides were then desalted for 10 min at a flow rate of 4 l/min. The trapped peptides were then separated using a 200-mm homemade microcapillary column consisting of C18 Aqua resin (3- m particle size; Phenomenex, Torrance, CA, USA) packed into 100 m of silica tubing with an orifice i.d. of 5 m.

An 11-step salt gradient was performed using 3 µl each of 0, 25, 50, 100, 250, and 500 mM ammonium acetate (in 0.1% formic acid/5% acetonitrile [ACN]) and 4, 5, 9, and an additional 9 µl of 500 mM ACN, (0.1% formic acid/30% ACN). Mobile phases A and B consisted of 0 and 100% ACN, respectively, each containing 0.1% formic acid. The LC gradient began with 5% B for 1 min and was ramped to 20% B over 5 min, to 55% B over 90 min, to 95% B over 1 min, remaining at 95% B for 13 min and then returning to 5% B over 5 min. The column was re-equilibrated with 5% B for 15 min before the next run. The electrospray voltage was set to 2.0 kV. During the chromatographic separation, the LTQ Orbitrap Elite was operated in data-dependent mode. MS data were acquired using the following parameters: five data-dependent

collision-induced dissociation–high-energy collision dissociation (CID-HCD) dual MS/MS scans per full scan; CID scans were acquired in the linear trap quadrupole (LTQ) with two-microscan averaging; full scans and HCD scans were acquired in the Orbitrap at resolution 60,000 and 15,000 respectively, with two-microscan averaging; 35% normalized collision energy (NCE) in CID and 45% NCE in HCD; and ±1-Da isolation window. Previously fragmented ions were excluded for 60 s. In CID-HCD dual scans, each selected parent ion was fragmented first by CID and then by HCD.

5. Protein identification and quantification

MS/MS spectra were analyzed using the following software analysis protocols with searching against the UniProt human database (UNIPROT.HUMAN. 2.4.2015). Reversed sequences of all proteins were appended into the database for calculation of false-discovery rates. ProLucid[14] software was used to identify the peptides using precursor and fragment ion mass errors of 25 and 600 ppm, respectively. Trypsin was selected as the enzyme, with three potential missed cleavages. TMT modification (+ 229.1629) at the N-terminus, addition of a lysine residue by the labeling reagent, and carbamidomethylation of cysteine were chosen as static modifications. Oxidation of methionine was chosen as a variable modification. CID and HCD tandem MS spectra from the same precursor ion are often combined by the software to allow for better

peptide identification and quantification.[15] We used software developed in-house in which reporter ions from an HCD spectrum were inserted into the CID spectrum with the same precursor ion as the previous scan. Reporter ions were extracted from small windows (±20 ppm) around their expected m/z value in the HCD spectrum. Output data files were filtered and sorted to compose the protein list using DTASelect[16] (The Scripps Research Institute, La Jolla, CA, USA), with ≥2 peptide assignments required for protein identification and a false-positive rate of <0.01.

A quantitative analysis was conducted using Census in the IP2 pipeline (Integrated Proteomics, San Diego, CA, USA). The intensity at a reporter ion channel for a protein was calculated as the average of the reporter ion's intensities from all constituent peptides derived from the identified protein.[2] The measured intensity ratios of proteins were transformed to the log2 scale. Ratios were averaged and proteins showing differences with $P < 0.05$ were defined as differentially regulated.

6. Statistical analysis

Data were analyzed by Student's t-test using SPSS software (SPSS Inc., Tokyo, Japan). The results are presented as mean ± standard deviation (SD). Differences are considered to be statistically significant when $P < 0.05$.

III. RESULTS

1. Increased proliferation of HDFs in stretched group during cell culture

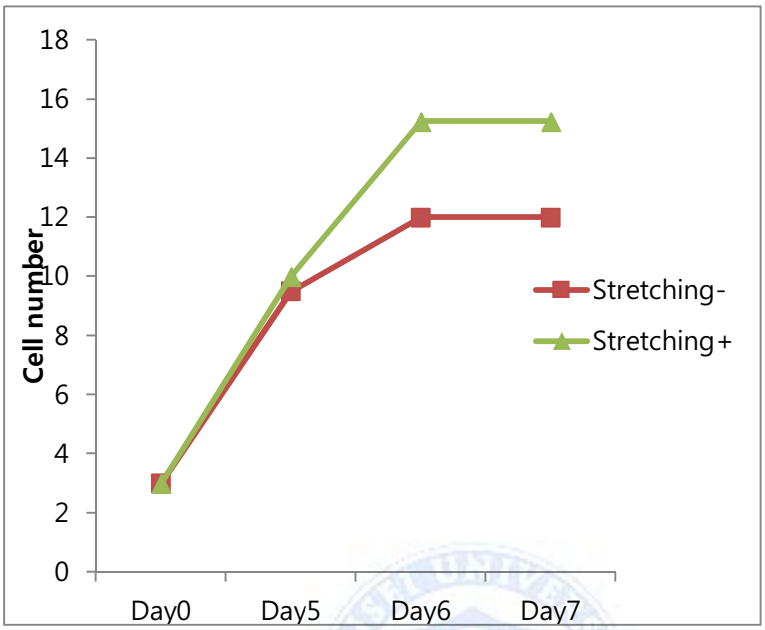
HDFs cell cultures was underwent for one week with mechanical stretching of 1 hours per day at 37 ℃, 5% CO2 for 3 days, and time-lapse recording was done for the process of cell cultures. During the cell cultures, the difference in proliferation over time with and without mechanical stretching was compared, and stretch loaded HDFs proliferated more than stretch nonloaded HDFs. While starting the culture by the same amount (3×10^4), stretched group showed 15×10^4 in cell counting after one week of culture, and nonstretched group showed 12×10^4 in cell counting. Consequently, the stretched group proliferated 1.27 times more than the nonstretched group (Figure 2). In total protein quantification, stretched group weighed 581ug after one week of culture, and nonstretched group weighed 171ug after one week of culture. The increase of total amount of proteins was greater than the increase of cell number in stretched group. The difference in proliferation can be noticed in microscopically in Figure 3. The number of stretched HDFs was increased and the stretched HDFs showed thicker morphology compared to nonstretched HDF.

Table 1. HDFs cell count change in time-lapse recording

	Day0	Day5	Day6	Day7
Stretched HDFs	3	9.5	12	12
Nonstretched HDFs	3	10	15.25	15.25

(x 10^4) ; HDF culture started with 3x 10^4 cells/well

A)



B)

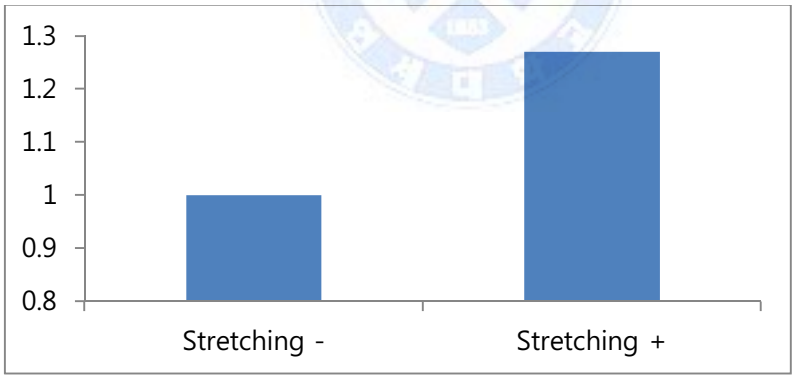


Figure 2. Difference in proliferation during the cell culture. A) HDF culture started with 3x 10^4 cells/well and stretched group showed 15 x 10^4 in cell counting and nonstretched group showed 12 x 10^4 in cell counting after one week of culture. B) Consequently, the stretched group proliferated 1.27 times more than the nonstretched group.

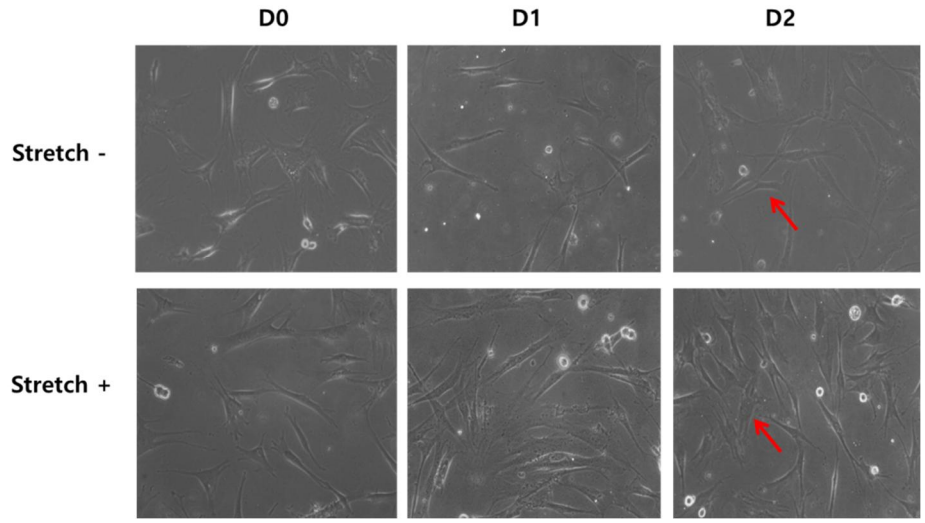


Figure 3. Change of HDFs in time-lapse recording. Increased proliferation of HDFs can be observed after stretching compared to nonstretched group. And stretched HDFs showed thicker morphology (arrow)

2. Quantitative proteomic analysis using a TMT-labeling method

Proteins extracted from stretch loaded HDFs and those from stretch non-loaded HDFs were individually labeled with TMTs for quantitative proteomic analysis. Samples were combined, speed-vacuum dried, and then dissolved in 50 l of water containing 0.1% formic acid for 2D-LC-MS/MS analysis. Initially, we identified 1415 proteins in the stretched and nonstrerched HDFs. We then attempted to identify proteins that either increased or decreased in expression level over time after mechanical stretch. Proteins for which the log2 ratio was statistically significant ($P < 0.05$; ratio ≤ -0.4 or ≥ 0.4) were considered to be differentially expressed. A total of 27 proteins were identified as candidates for further investigation. Among these proteins, 16 proteins were upregulated and 11 proteins were downregulated. Specific information about the description and log2 ratio are shown in Table 2 and Table 3.

Table 2. Upregulated proteins identified using the TMT labeling method.

NO.	LOCUS	DESCRIPTION	ratio log2 (stretched : nonstretched)
1	sp|Q9HB40|RISC_HUMAN	SCPEP1 Retinoid-inducible serine carboxypeptidase	0.41
2	sp|Q05682-5|CALD1_HUMAN	CALD1 Isoform 5 of Caldesmon	0.41
3	sp|O94925|GLSK_HUMAN	GLS Glutaminase kidney isoform, mitochondrial	0.41
4	sp|P42765|THIM_HUMAN	ACAA2 3-ketoacyl-CoA thiolase, mitochondrial	0.41
5	sp|P02788|TRFL_HUMAN	LTF Lactotransferrin	0.47
6	sp|O95394|AGM1_HUMAN	PGM3 Phosphoacetylglucosamine mutase	0.47
7	sp|O60486|PLXC1_HUMAN	PLXNC1 Plexin-C1	0.48
8	sp|P48163|MAOX_HUMAN	ME1 NADP-dependent malic enzyme	0.51
9	sp|P02765|FETUA_HUMAN	AHSG Alpha-2-HS-glycoprotein	0.54
10	sp|P17301|ITA2_HUMAN	ITGA2 Integrin alpha-2	0.54
11	sp|P01023|A2MG_HUMAN	A2M Alpha-2-macroglobulin	0.55
12	tr|C9JFR7|C9JFR7_HUMAN	CYC Cytochrome c (Fragment)	0.56
13	tr|K7EQ73|K7EQ73_HUMAN	DNAJC7 DnaJ homolog subfamily C member 7 (Fragment)	0.58
14	tr|F5H7Y0|F5H7Y0_HUMAN	PDLIM5 PDZ and LIM domain protein 5	0.58
15	sp|P15121|ALDR_HUMAN	AKR1B1 Aldose reductase	0.74
16	sp|P04179-4|SODM_HUMAN	SOD2 Isoform 4 of Superoxide dismutase	0.76

Table 3. Downregulated proteins identified using the TMT labeling method.

NO.	LOCUS	DESCRIPTION	ratio log2 (stretched : nonstretched)
1	sp|P02452|CO1A1_HUMAN	COL1A1 Collagen alpha-1(I) chain	-0.59
2	sp|P07093-3|GDN_HUMAN	SERPINE2 Isoform 3 of Glia-derived nexin	-0.58
3	sp|Q99715|COCA1_HUMAN	COL12A1 Collagen alpha-1(XII) chain	-0.55
4	sp|Q5JRA6|MIA3_HUMAN	MIA3 Melanoma inhibitory activity protein 3	-0.54
5	sp|P12110|CO6A2_HUMAN	COL6A2 Collagen alpha-2(VI) chain	-0.51
6	tr|A0A087X0S5|A0A087X0S5_HUMAN	COL6A1 Collagen alpha-1(VI) chain	-0.49
7	sp|Q9ULD0-3|OGDHL_HUMAN	OGDHL Isoform 3 of 2-oxoglutarate dehydrogenase-like, mitochondrial	-0.47
8	sp|Q52LR7|EPC2_HUMAN	EPC2 Enhancer of polycomb homolog 2	-0.45
9	tr|A0A087WTA8|A0A087WTA8_HUMAN	COL1A2 Collagen alpha-2(I) chain	-0.45
10	sp|Q8NB90|SPAT5_HUMAN	SPATA5 Spermatogenesis-associated protein 5	-0.42
11	tr|H0Y8C4|H0Y8C4_HUMAN	PPP2R5D Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform (Fragment)	-0.40

IV. DISCUSSION

Since it was revealed that the mechanical tension is important in the development of pathologic scars such as keloids and hypertrophic scar, there have been many studies to find out the effect of mechanical tension to the pathogenesis of keloids. One of the previous studies evaluated the change in RNA expression of HDFs after mechanical stretching using DNA microarray analysis and showed the differences in integrin signaling genes and Wnt signaling genes but there was no difference in TGF β-signaling molecules.[17]

In our study, we examined the difference of protein expression according to the presence or absence of stretching. To the best of our knowledge, this study is the first study which showed the effect of multidirectional mechanical stretching on protein expression of HDFs using proteomics.

In our study, we found 27 differently expressed proteins between stretched HDFs and nonstretched HDFs. Among them, 16 proteins were upregulated and 11 proteins were downregulated in stretched HDFs.

As a result of the review of the literature about the differently expressed proteins, we could find the several clues about the difference in protein expression according to the functions of the proteins. For examples, some of the proteins were related to the cell metabolism and oxidative stress, or cell proliferation and cell cycle regulation.

Among the upregulated proteins, SOD2 (Isoform 4 of Superoxide dismutase) was the most highly upregulated protein. It has the function of transforming toxic superoxide into hydrogen peroxide and diatomic oxygen. It clears reactive oxygen species (ROS) to protect against cell death. SOD2 is related to proliferative feature of tumor. SOD2 is also related to ageing, cancer, and neurodegenerative disease and one previous study revealed that the overexpression of SOD2 has been linked to increased invasiveness of tumor metastasis.[18,19] And another study reported that the eruptive keloids were associated with the breast cancer.[20]

AKR1B1 (aldose reductase) was the second most highly upregulated protein. Aldose reductase is a key enzyme in the polyol pathway and it is highly associated with ROS formation in human tissues. It catalyzes nicotinamide adenosine dinucleotide phosphate (NADPH) dependent reduction of glucose to sorbitol, leading to excessive accumulation of intracellular ROS in various tissues.[21] According to the one recent study, TGFβ1 activated NADPH oxidase, resulting in ROS generation. And activated HDFs express functionally active NADPH oxidase, thus HDFs are also an important source of ROS in the human skin.[22]

Oxidative stress in scar pathogenesis is becoming increasingly important in these days. The two most highly increased proteins in this study are both ROS and metabolism related proteins, suggesting that ROS and oxidative stress

have a critical role in scar developing process related to the mechanical stretching or skin tension.

In addition, siRNA of NOX2, a membrane-bound subunit of NADPH oxidase, inhibited collagen synthesis in keloid dermal fibroblast in the previous study, suggesting that NADPH could serve as a potential therapeutic target for patients with keloid.[22] Aldose reductase which is one of the highly upregulated proteins in our study could also be a potential therapeutic target for keloid treatment. Its role in ROS formation in human tissues is increasingly important and aldose reductase inhibitor is already considered as an emerging therapeutic strategy in preventing cardiovascular complications such as ischemia/reperfusion injury, atherosclerosis, atherothrombosis, and DM complications.[21]

DNAJC7 (DnaJ homolog subfamily C member 7 (Fragment)) is also upregulated in our study. It belongs to the heat shock protein 40 family and is considered to be related to cell cycle regulation. It interacts with RAD9A, which is the cell cycle checkpoint control protein.[23]

Although it is well known that TGFβ1 plays an important role in scar mechanotransduction,[24,25] TGFβ1 expression was not upregulated in this study, and this is the same result with the previous report about mechanical stretching on HDFs.[17] However, PMLIM5 which is known to be associated with TGF β−signaling was upregulated after stretching in our study.[17]

PDLIM5 (PDZ and LIM domain protein 5) is also related to cytoskeleton organization, cell lineage specification, organ development, and oncogenesis. One previous study showed that the knockdown of PDLIM5 suppressed gastric cancer cell proliferation in vitro.[26] This protein interacts with PRKCB1 (protein kinase C beta 1) and is involved in apoptosis induction and endothelial cell proliferation.[27] And one genomic study by Reichenbach et al. also showed increase of gene expression of PDZ and LIM domain 7 in response to mechanical stretch in HDFs.[28]

Furthermore, ITGA2 (Integrin alpha-2) was upregulated after stretching in our study. This result is the same with the previous study, and this suggests that integrin signaling pathways participated in stretching induced mechanical transduction in scar development.[17]

Among the downregulated proteins, PPP2R5D (Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform (Fragment)) plays a role in negative control of cell growth and division. In previous study suggested that the mutations in PPP2R5D caused human overgrowth.[29]

SERPINE2 (Isoform 3 of Glia-derived nexin) is also the regulator of cancer cell growth and neo-angiogenesis.[30] Wang et al. reported that the upregulated SERPINE2 may contribute to the aggressive gastric cancer[31] and François et al reported that the increased expression is associated with idiopathic pulmonary fibrosis.[32]

In this study, collagen synthesis seemed to be downregulated in stretched HDFs. Generally, the development and progression of keloids is considered to correlate closely with local skin tension. And tension-reduction methods have long been effective in the clinical prevention and treatment of keloids. However, our results showed that the mechanical stretching did not directly increase the collagen synthesis by fibroblasts. It was same results with the previous study which demonstrated the effect of mechanical stretching on HDFs using microarray.[17] Paradoxically, many collagens seemed to be downregulated in stretched HDFs in our study. However, the increase of total amount of proteins was greater than the increase of cell number in stretched group. Thus, it is possible that other ECM synthesis was much more increased than the collagen synthesis, thus the ratio of collagen seems to be reduced in stretched HDFs. And it remains possible that other ECM synthesis has to be preceded or certain thresholds have to be crossed before accumulation of the collagen.

V. CONCLUSION

The stretched HDFs proliferated greater than the nonstretched HDFs and the increase of total amount of proteins was greater than the increase of cell number in stretched group. In this study, quantitative proteomic analysis using a TMT-labeling method revealed 27 differently expressed proteins between stretched and nonstretched group. Among them, 16 proteins were upregulated and 11 proteins were downregulated in stretched group compared to nonstretched group. Some of the differently expressed proteins were associated with cell metabolism and oxidative stress, or cellular proliferation and cell cycle regulation. In the future, validating study will be needed for functional verification of each protein and evaluating the association with the pathway of keloid development. And a better understanding of the effects of mechanical stretch on human fibroblasts will help the development of novel technologies that can prevent or reduce pathological scar formation in the future.

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ABSTRACT (IN KOREAN)

피부 섬유아세포에서의 기계적 장력의 영향

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신성식

기계적 장력은 흉터 발생에 중요한 영향을 주며, 이것은 켈로이드 및 과증식 흉터가 장력이 많이 걸리는 부위에 호발하는 것을 통해 알 수 있다. 또한 기존의 연구에서 기계적 장력이 섬유아세포를 활성화시켜 콜라겐 합성 및 증식을 일으키고, 섬유화에 중요한 역할을 하는 근섬유아세포로의 변화를 일으키는데 중요한 역할을 하는 것으로 밝혀졌다.

따라서 본 연구의 목적은 기계적 장력이 피부 섬유아세포에 끼치는 영향을 알아보기 위해 프로테오믹스 방법을 통해 장력의 유무에 따른 단백질 발현의 차이를 알아보고자 하였다.

기계적 장력을 생성하기 위해 우리는 진공을 이용한 프로그램화된 시스템을 만들었다. 이 장치는 다 방향의 장력을 제공하며, 부착된

세포에 대해 실시간 조작이 가능하였다. 기계적 장력은 3일간 2시간씩 적용하였다. 1주일 간의 세포 배양 후, 장력의 유무에 따른 피부 섬유아세포의 단백질 발현의 차이를 알아보기 위해 프로테오믹스 분석을 하였다.

장력을 준 그룹에서 피부 섬유아세포가 더 많이 증식하였고, TMT-labeling 방법을 통한 단백질 분석에서 두 그룹간에 통계적으로 유의한 발현의 차이가 있는 27개의 단백질을 발견하였다. 이 중 16개의 단백질은 장력을 준 그룹에서 발현이 증가하였고, 11개의 단백질은 장력을 준 그룹에서 발현이 감소하였다.

향후 각 단백질의 기능 분석 및 켈로이드 발생기전과의 연관성에 대해 분석하는 연구가 필요하겠고, 피부 섬유아세포에 대한 기계적 장력의 영향에 대한 이해도가 증가함에 따라 병적 흉터의 발생의 예방 및 감소를 위한 새로운 기술의 발전도 기대할 수 있을 것이다.

핵심되는 말: 피부 섬유아세포, 기계적 장력, 켈로이드, 흉터 발생, 프로테오믹스