学 位 論 文 の 要 旨

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学 位 論 文 名	Cell Cycle Perturbation Induces Collagen Production in Fibroblasts
発 表 雑 誌 名 （巻，初頁～終頁，年）	International Heart Journal (In Press)
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論 文 内 容 の 要 旨

INTRODUCTION

Cardiac fibroblasts are activated in the area of a myocardial infarction (MI) in the heart, producing collagen or other extracellular matrixes (ECM). Fibrotic scars replace the damaged cardiomyocytes and support the structural integrity of the heart, in a manner called "reparative" or replacement fibrosis. At the same time, cardiac fibroblasts can be inappropriately activated in hypertensive or diabetic patients, leading to "reactive" or excessive fibrosis. As inappropriate accumulation of ECM can restrict ventricular function or elicit cardiac arrhythmias, ECM is currently becoming an established therapeutic target in heart diseases. Anti-fibrotic therapy in the treatment of heart diseases targets excessive fibrosis while leaving replacement fibrosis unaffected. Hence, it is crucial to understand the molecular processes by which fibroblasts are activated in an ischemic area. TGF-β is a well-known profibrotic factor which increases the transcript levels of type I collagen, leading to the accumulation of ECM. Moreover, TGF-β activates cardiac fibroblasts and acts as a master cytokine in ECM production. However, knowledge of fibroblast activation signals other than TGF-β remains incomplete. Decreased vascular flow at the MI area strikingly reduces tissue oxygen tension or nutrient levels. We have previously reported the effects of decreased vascular flow during scar formation, and showed that tissue hypoxia activates the fibroblasts in the lung.

Here, we found that serum deprivation, which reflects one of the characteristics of the

tissue microenvironment of the MI area, decreases the transcript levels of cell cycle-related genes and strikingly increases collagen production in fibroblasts. The facilitation of collagen synthesis is independent of the transcript levels of type I collagen genes. The results uncover a previously undescribed link between cell cycle arrest and collagen synthesis.

MATERIALS AND METHODS

Murine mesenchymal C3H10T1/2 cells were cultured in growth media with or without 10% FBS (Fetal Bovine Serum) for 24 hours. Pharmacological studies were prepared by incubating with 4mM double thymidine block and methotrexate 10nM in DMEM with FBS.

Total RNA was isolated from cells. Single-strand cDNAs were synthesized total RNA. The quantitative PCR was performed in the LightCycler system. All results from quantitative PCR were calculated relative to 18S rRNA (18S ribosomal RNA) as a normalization control.

Total RNA was collected for transcriptome analysis (RNA-seq). Single-end RNA-seq libraries were prepared. Sequencing runs were performed on an Illumina Genome Analyzer IIx. Generations of gene expression data, normalization and gene annotation processes were performed. For evaluation of gene expression, the normalized expression value(NE-value) was calculated as follows: the number of reads per a gene $\times 10^7$/ the number of mapped reads in the genome $\times$ gene length. Gene ontology enrichment analysis was carried out using DAVID software tools.

All results are presented as means $\pm$ standard deviation (SD). Two-tailed, unpaired Welch's t-tests were applied to compare the two groups. Statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

In this study we found that a serum-starved environment induces collagen production in C3H/10T1/2 cells. Facilitation of collagen synthesis is mediated through a process independent of the transcriptional levels of type I collagen genes. Pharmacological experiments showed that cell cycle perturbation directly accelerates collagen synthesis in fibroblasts. While the results uncovered a link between the cell cycle and fibroblast activation that was previously not described, it remains unclear how cell cycle arrest induces collagen production in fibroblasts. As energy substrate utilization is known to affect a cellular function, it may account for the acceleration of collagen production in cell cycle-arrested fibroblasts. Glycine is the most abundant amino acid in the collagen peptide chain. Importantly, glycine is also used for the biosynthesis of purine nucleotides, which are essential for the proliferation of C3H/10T1/2 cells. Therefore, we speculate that collagen synthesis and cell proliferation may act antagonistically with regard to the utilization of glycine and potentially other energy substrates. Inhibition of the

cell cycle may redirect the intracellular use of glycine away from cell proliferation and towards collagen synthesis. MTX induces cell cycle arrest and is clinically used to treat patients with rheumatoid arthritis. Pulmonary fibrosis is one of the adverse effects observed in patients treated with MTX. In addition, several antineoplastic agents, including bleomycin and irinotecan, or radiation therapy can elicit pulmonary fibrosis. The results in this study illuminate a previously undescribed pathway which may have implications to chemotherapy or radiation-induced organ fibrosis. Management of excessive tissue fibrosis is crucial to maintain the homeostasis of the organs. In order to establish anti-fibrotic therapy for heart diseases, we need to target the malignant excessive fibrosis specifically, while leaving the reparative replacement fibrosis unaffected. Exploring the molecular processes in fibroblast activation will allow us to better understand the roles of tissue fibrosis in organ integrity.

CONCLUSION

Based on transcriptomic and pharmacological studies, we found that cell cycle perturbation is directly links to collagen production in fibroblasts. Importantly, collagen synthesis is increased independently of the transcriptional levels of type I collagen genes. These results uncover a novel mode of fibroblast activation in the ischemic area, which will help us to gain a better understanding of the molecular processes involved in cardiac fibrosis.