GENERATION OF TRANSGENIC MICE EXPRESSING FRET-BASED TENSION SENSOR: MASUREMENT WITH CONVENTIONAL CLSM

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Introduction

To visualize forces at the molecular level, several tension sensors have been developed using Förster resonance energy transfer (FRET) and applied in cultured cells [1-2]. However, it is very difficult to introduce these sensors in tissues. Recently, Tao *et al*. [3] developed transgenic mice expressing a tension sensor to visualize forces at the tissue level. Their used a high-cost FLIM (Fluorescence Lifetime Imaging) system to measure FRET and have not reported change in FRET in tissues with conventional CLSM (confocal laser scanning microscope). Furthermore, the fluorescent protein pair adopted in their sensor cannot be observed with widely-used 488 nm laser system. In a previous study, we developed a tension sensor by inserting a FRET cassette sstFRET-GR into actinin [4]. The FRET cassette is made of two fluorescent proteins EGFP and mCherry connected with spider silk protein. This sensor is bright enough and the tension applied to it can be evaluated with a FRET ratio that is the ratio of acceptor (mCherry) to donor (EGFP) fluorescence using a conventional 488 nm CLSM system. In this study, we introduced the gene of this sensor into the C57BL/6N mice to obtain mice expressing the tension sensor.

Methods

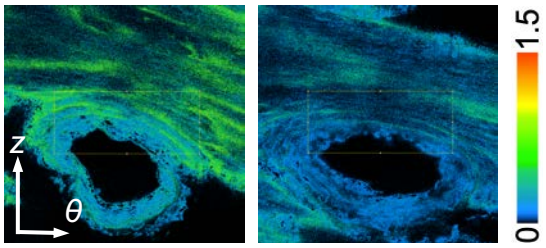
We introduced the gene of our actinin tension sensor engineered with the Cre/loxP system into the ROSA26 locus of the C57BL/6N mice, and crossbred them with Cre mice to obtain mice expressing the tension sensor. We excised various tissues, including the aorta and tendon, and isolated cells from each tissue with enzymes. The isolated tissues and cells were stretched with a tensile tester (STB 150W NK, Strex, Japan) under a conventional CLSM (LSM880, Carl Zeiss, Germany) with a 63× oil immersion objective at room temperature in phosphate buffered saline (PBS). The fluorescence of EGFP and mCherry was obtained under the excitation with a 488 nm wavelength laser at each stretch step to calculate the FRET ratio.

Results

The fluorescence was so bright that the change in the FRET ratio was observable with a general confocal microscope (Figure 1). We performed tensile tests of aortic tissues and confirmed that the FRET ratio decreased in response to stretch as expected (Figure 2). Interestingly, the decrease in the normalized FRET ratio was ~10% at 10% stretch for aortic tissues and tendons, while that of smooth muscle cells (SMCs) isolated from aortic tissue was ~20% at 10% stretch.

Discussion

It has been reported that strain in actin stress fibers in SMCs in the direction of the fiber axis was half of the strain in the smooth muscle layers in the circumferential direction of the aorta [5]. The relatively low FRET ratio change in aortic tissues than SMCs observed in this study may support this observation. The present FRET mice may become a powerful tool in the mechanobiology of cells and tissues.



(a) 0% (b) 26.5%

Figure 1: Change in FRET ratio map of aortic tissue in response to stretch in the circumferential direction.

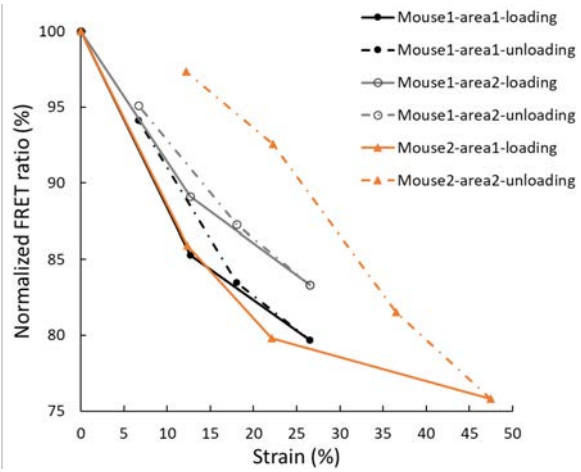


Figure 2: Change in FRET ratio of aortic tissues normalized with the FRET value just before stretch.

References

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