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The exploration of constitutively expressed myokines utilizing tissue-engineered skeletal muscle

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Objective Recent evidence has identified skeletal muscle as a secretory organ. Many myokines, which are bioactive substances secreted from skeletal muscle, have been identified in plane muscle culture cells. Compared to the plane muscle culture cells, the tissue-engineered muscle is an excellent model as culture system mimicked native skeletal muscle. However, constitutively expressed genes and secreted compounds from tissue-engineered muscle have not been analyzed sufficiently. The purposes of this study were 1) to clarify kinetics of constitutively secreted compounds, and 2) to explore constitutively expressed genes in the tissue-engineered muscle.

Methods C2C12 cells embedded within collagen gel solution were placed between two tendons made up of elastase-treated acellular porcine blood vessel. The constructs were cultured in growth media for 2 days and cultured in differentiation media for 6 days. To compare with plane culture cells, C2C12 cells were cultured in plane under the same condition as the construct. The culture media were obtained, and analyzed by MALDI-TOF Mass Spectrometry. Furthermore, constitutively up-regulated genes in tissue-engineered skeletal muscle were explored based on microarray analysis and confirmed by RT-PCR.

Results MALDI-TOF Mass Spectrometry revealed that the number of detected peaks in tissue-engineered muscle was abundant compared to that of plane muscle culture cells, especially at range of low molecular weight. Furthermore, the detected peaks were substantially different among these culture media and specific peaks were identified in tissue-engineered muscle. Based on microarray analysis, the transcription of cholecystokinin identified, and confirmed the up-regulation in tissue-engineered skeletal muscle by RT-PCR.

Conclusions These results suggested that the tissue-engineered muscle constitutively secreted many compounds compared to plane culture cells, especially at range of low molecular weight. Furthermore, the transcription of cholecystokinin was up-regulated in tissue-engineered skeletal muscle. Besides of the plane muscle culture cells, it is possible to expect to obtain novel myokines utilizing tissue-engineered muscle.