論 文 要 旨 等 報 告 書

氏　　名	Sakhr Ahmed Murshid
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学位論文題名	Actin and microtubule cytoskeletons of the processes of 3D-cultured MC3T3-E1 cells and osteocytes（3次元培養されたMC3T3-E1細胞と骨細胞の突起におけるアクチンおよび微小管細胞骨格について）
論文審査委員	教授 山城　隆　助教授 池亀 美華　教授 杉本 朋貞

学 位 論 文 内 容 の 要 旨

【INTRODUCTION】 It is reported that 3D culture of the osteoblast cell line, MC3T3-E1; primary osteoblasts; and the osteocytic cell line MLO-Y4 all develop a common morphology, including stellate-like shape and cytoplasmic projections; closely resembling those of osteocyte processes *in vivo*. Despite the differences in morphology between osteoblasts and osteocytes in 2D culture, osteoblasts in 3D culture could form dendritic processes like those of osteocytes. We previously examined the relative distribution of actin filaments, actin binding proteins and microtubules in osteoblasts and osteocytes in 2D culture. In the present study, cytoskeletal structures of MC3T3-E1 processes and osteocyte processes were examined for the first time in 3D culture. In addition, we examined the relative importance of the cytoskeletal elements for process integrity and process formation in 3D-cultured MC3T3-E1 cells and osteocytes.

【MATERIALS AND METHODS】 We used primary chick osteocytes and osteoblast cell line, MC3T3-E1 cells. 2D- and 3D-cultured osteocytes and MC3T3-E1 cells were prepared. Immunofluorescence staining was performed for actin, fimbrin, alpha-actinin, myosin, tropomyosin and microtubules. Next, 3D-reconstructions of fluorescent images were visualized with IMARIS software. Furthermore, cytochalasin D, an actin-disrupting agent, and nocodazole, a microtubule disrupting agent were used.

【RESULTS】 Fluorescence staining of actin showed osteocyte processes, in both 2D and 3D cultures, were actin-rich structures. Interestingly, the number of processes per cell does significantly differ ($P<0.05$) between 2D- (8.5±0.781) and 3D- (12.75±0.616) cultured osteocytes. MC3T3-E1 cell in 3D culture developed actin-rich

processes and branches similar to those seen in 2D- and 3D-cultured osteocytes. Immunofluorescence staining of fimbrin showed their presence in the entire length of the processes of 3D-cultured osteocytes and MC3T3-E1 cells. Fimbrin was found to have a different distribution pattern in 3D cultured osteocyte processes and MC3T3-E1 processes. Anti-fimbrin immunoreactivity in 3D-cultured osteocyte processes was appeared as thick bundles, whereas in 3D-cultured MC3T3-E1 showed sparse and periodic distribution. Anti-alpha-actinin was localized only in the proximal one third or one half of the processes of 3D-cultured osteocytes and MC3T3-E1 cells. The distribution pattern of alpha-actinin was similar in both cell types and appeared as serial dots in the processes. Myosin localized in the entire length of the processes of 3D-cultured osteocytes and MC3T3-E1 cells. Myosin immunoreactivity in 3D-cultured osteocyte processes was appeared as periodic segments, whereas in 3D-cultured MC3T3-E1 processes showed sparse and periodic distribution. Tropomyosin in 3D-cultured osteocytes and MC3T3-E1 cells was observed throughout the processes. The distribution pattern of tropomyosin was similar in both cell types and appeared as dense serial dots in the processes. Interestingly, the difference in the distribution of microtubules in the processes of 3D-cultured osteocytes and MC3T3-E1 cells was obvious. Microtubules extended only into the proximal one fourth of osteocyte processes. In contrast, microtubules extended into the full length of processes as well as into the branches of MC3T3-E1 cells. In 3D-cultured osteocyte, the actin cytoskeleton is important for processes integrity and formation, whereas in 3D-cultured MC3T3-E1 cells, microtubules are important for processes integrity and formation.

【DISCUSSION】 Osteocytes maintain their morphology and organization of their actin cytoskeleton, which is essential for their integrity, in their processes under all culture conditions. In contrast, MC3T3-E1 cell process integrity in 3D culture depends on microtubules rather than actin. High expression of fimbrin in osteocyte processes may relate to higher stability than those in MC3T3-E1 cell processes in 3D collagen gel. High expression of myosin in MC3T3-E1 cell processes may relate to higher dynamic and motile than osteocyte processes in 3D collagen gel. We believe that these differences in the cytoskeleton in the processes of 3D-cultured MC3T3-E1 cells and in the cytoskeleton of the dendrites of osteocytes mean that the cytoskeletons in each type of process drive different functions, as the latter utilize their processes as mechanosensors which influence bone remodeling.

論文審査の結果の要旨

本研究では、３次元培養した骨芽細胞株 MC3T3-E1(以下骨芽細胞)と単離した初代培養骨細胞のアクチン細胞骨格および微小管細胞骨格の分布を比較した。その結果、アクチンおよびアクチン結合蛋白である、fimbrin、α-actinin、myosin、tropomyosin は、骨芽細胞および骨細胞の両者において、すべての突起に観察された。しかし、fimbrin の発現は、骨細胞に強く表れる一方、myosin は、骨芽細胞の突起に強く表れた。一般に、fimbrin は形態の安定化に関与していることから、骨細胞の突起は３次元培養中でも安定した構造であることが考えられた。また、myosin はアクチン線維の活発な動きに関与していることから、骨芽細胞の突起は伸展・収縮していることが考えられた。さらに、微小管蛋白の分布を検出したところ、骨芽細胞の突起には全長に亘り微小管が走行しているのに対して、骨細胞では、細胞体側のわずかな領域のみに存在していた。以上より、骨芽細胞および骨細胞両者の突起を裏打ちする細胞骨格が異なることが明らかとなり、機能的にも異なることが示唆された。次に、突起形態の維持にアクチンあるいは微小管のどちらが関与しているのかを検討するために、３次元培養を開始し、突起が十分に形成されたのちに、アクチン脱重合剤を用いてその影響をみた。結果、骨芽細胞の突起は、なんら変化をみせなかったのに対し、骨細胞の突起は、著しく収縮し、一方、微小管脱重合剤により、骨芽細胞の突起は、収縮を生じ、骨細胞の突起にはなんら影響がなかった。さらに、突起形態の形成過程に関与する蛋白を検討するために、３次元培養を開始すると同時にアクチン脱重合剤を用いてその影響を観察した。その結果、骨芽細胞では、突起の数が減少し、長く伸びた突起が形成された。しかし、骨細胞は、突起を全く形成しなかった。一方、微小管脱重合剤により、骨芽細胞の突起は、全く形成されなかったが、骨細胞では突起が形成され、その形態は未処置群と同様であった。これらの結果から、３次元培養された骨芽細胞では、突起の形成と維持に微小管が重要であり、骨細胞ではアクチン細胞骨格が重要であると考えられた。

本研究は、骨芽細胞と骨細胞の３次元的細胞骨格の分布を明らかにしたのみならず、２種細胞におけるアクチンと微小管の役割差異を実験的に証明したものであり、博士(歯学)の学位論文に値するものと認めた。