APPLICATION NOTE

A1R HD25, AX/AX R Confocal Microscope

Macro to micro skeletal muscle fiber formation mechanism captured with large FOV confocal microscope

Skeletal muscle formation begins with myoblast differentiation by expression of master transcription factors (Pax7, MyoD, Myogenin) in mesenchymal stem cells. Subsequently, molecules such as Myomaker and Myomixer fuse (multinucleate) myoblasts and mature them into skeletal muscle fibers. When forming skeletal muscle fibers, mononuclear myoblasts reorganize their cytoskeleton, consisting of actin, tubulin, etc., and show an elongated morphology. At higher densities, these elongated cells align with each other to form a locally ordered phase. When this cell population is observed from a macro viewpoint, it shows a characteristic spiral pattern, which is inherited by the subsequent skeletal muscle fibers. On the other hand, parts where the cell population is not well oriented are called topological defects with respect to the ordered phase and were observed as having an accumulation of mononuclear round cells. The spiral pattern formation of such myoblast populations may be important for subsequent multinucleation and maturation into skeletal muscle fibers.
Mr. Yoshizuki Fumoto, Dr. Tsukasa Oikawa, et al. at the Department of Molecular Biology, Hokkaido University Graduate School of Medicine, aim to elucidate the molecular mechanism that is a necessary condition for myoblast populations to form spiral patterns. To that end, they are challenging the question of how microscopic molecular dynamics such as transcription factors, membrane fusion molecules and cytoskeletal molecules relate to macroscopic phenotypes at the cell population and tissue level.
In this application note, we introduce the behavior of related molecules in cells and the macroscopic pattern of cell populations, as captured by a confocal microscope.

Keywords: Confocal microscope, Cell population, Myoblasts, Skeletal muscle fibers

Results

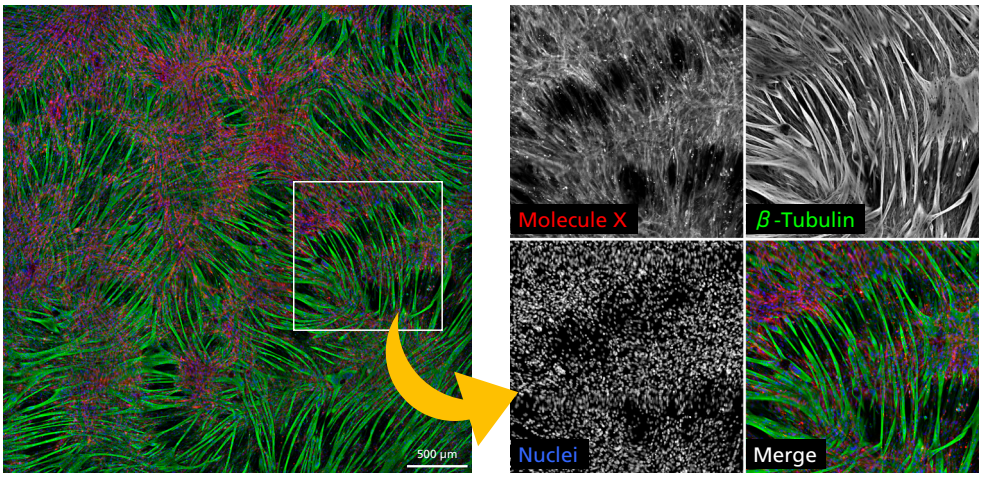

Figure 1. Populations of myoblasts, which form a spiral pattern, and localization of target molecule X
Immunofluorescence of mouse myoblast cell line C2C12 that was stained after fixation with methanol. β-Tubulin (Alexa 488: green), endogenous molecule X (Cy3: red), nuclei (TO-PRO3: blue)
Acquisition condition: Zoom; 0.7X, Image stitching; 5 x 5 images, Objective; Plan Apochromat Lambda 20X (NA 0.75)

Summary

This study benefited from a confocal microscope that provides a large FOV, which simultaneously enables observation of macroscopic cellular patterns and microscopic molecular behavior with high resolution.
Skeletal muscle cells show a spiral pattern when differentiated over a week. In this study, observation with the confocal microscope revealed that the expression of a target molecule X, which was not biased among cells before differentiation, is localized mainly in mononuclear cells in topological defects after differentiation, and not in skeletal muscle fibers that show a macroscopic spiral pattern. In addition, forced expression or knockdown of molecule X inhibited the formation of topological defects and the formation of skeletal muscle fibers. These results suggest that molecule X regulates the formation of locally ordered phases in myoblasts and, as a cell population, contributes to the formation of spiral patterns and skeletal muscle fibers. Analyzing the detailed functions of molecule X is expected to lead to the elucidation of the mechanism that connects microscopic molecular dynamics and macroscopic histogenesis.

Product Information

AX R Confocal Microscope

Supports high-speed, high-resolution, large field of view confocal imaging, with reduced phototoxicity and photobleaching

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (galvano) / 2K (resonant)
- High throughput: Ultra-wide field of view of 25 mm