

ANALYSIS FOR EFFECT OF CELL SHAPE ON HIPPO SIGNALING PATHWAY USING MICRO-FABRICATED CELL CULTURE PLATFORM

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ABSTRACT

Cell density affects Hippo signals, which regulates cell proliferation, but its molecular mechanism is still unclear. In this study, we examined the effect of cell shape on Hippo pathway by using micro fabricated cell culture platform and found that F-actin formation was modulated by cell shapes and F-actin had an inhibitory effect on Hippo pathway. This findings suggest that cell shape is an important factor in the regulation of cell proliferation via Hippo signaling, and that cells can detect density through their resulting morphology by F-actin mediated mechanism to regulate proliferation.

KEYWORDS

Micro-domain cell culture, Hippo signaling, Contact inhibition, Cell shape, F-actin/stress fiber.

INTRODUCTION

The Hippo signaling pathway is an evolutionally conserved tumor suppressor signaling pathway regulating cell proliferation. The core components of mammalian Hippo pathway consist of protein kinases Mst1/2, Lats1/2, co-activator proteins Yap/Taz, and transcription factors Tead1-4. In cultured cells, Hippo signal is involved in cell density-dependent regulation of cell proliferation, which is known as "cell contact inhibition of proliferation" [1,2]. At low cell density, weak Hippo signal allows nuclear accumulation of Yap, which promotes cell proliferation by activating Tead, in contrast, at high cell density, strong Hippo signal suppresses cell proliferation by inhibiting nuclear accumulation of Yap. However, the mechanisms by which cells sense cell density remains unknown.

Changes in cell density would alter both cell-cell contacts (adhesions) and cell shapes. Some studies suggested Hippo signals activation by cell-cell contact. Nevertheless, there is also a suggestive report showing alteration of cell proliferation by manipulating the shape of a single endothelial cell [3]. However, little is known about the role of cell shapes in regulation of the Hippo signal. In this study, we focused on the role of cell shape in regulation of Hippo signaling, and examined it by separating from the effects of cell-cell contacts/adhesions by manipulating the shape of a single cell using micro-fabricated cell culture platform.

EXPERIMENT

We first confirmed the correlation between the area covered by each cell (hereafter designated as 'cell area') and Yap distribution in mouse embryonic fibroblast NIH3T3 cells at various densities. The cell area was measured using the area of EGFP expressing cells sparsely mixed in the cultures. The cell area was smaller in high density condition than that of low density, and Yap was mostly in cytoplasmic and nuclei at high and low density, respectively (Fig. 1). Thus, difference in cell area (i.e. cell shape) is likely to affect Hippo signaling but its contribution is controversial because of existence of cell-cell contact.

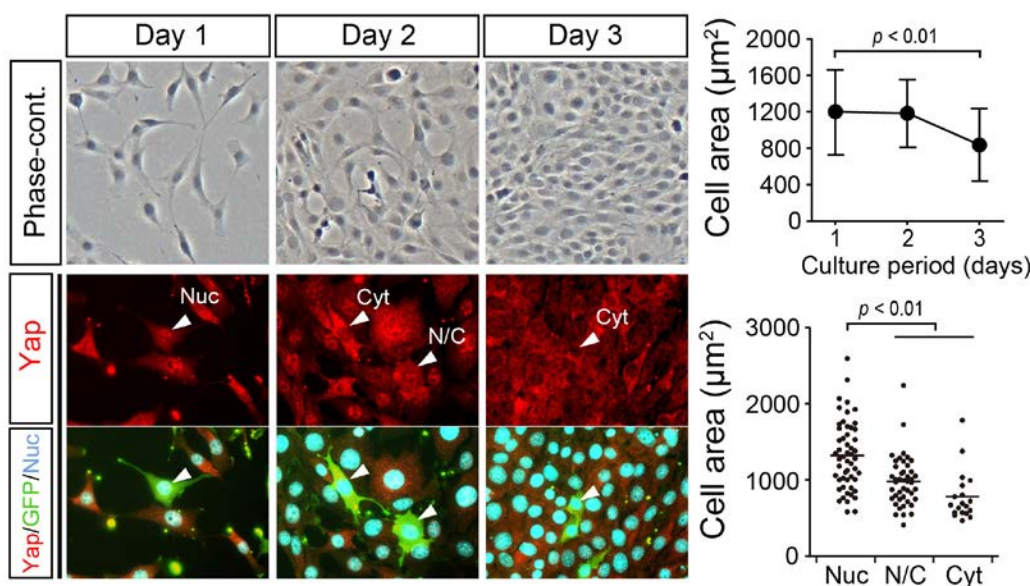


Fig. 1. Cell density affects cell shape and Yap subcellular localization pattern. In this study, Yap distribution patterns were classified as Nuc (nucleus > cytoplasm), Cyt (nucleus < cytoplasm), and N/C (diffuse).

Next, to clarify the role of cell shape, we fabricated small cell-adhesive areas termed microdomains on cell culture dish by photo-micropatterning based technique [4]. By culturing individual cells on various sizes of micro domains, we could control the shape of a single cell in the absence of cell-cell contacts (Fig. 2, left). In the small domains (e.g. 20 x 20 μm), cells were less-expand (round shape) and Yap was mostly in the cytoplasm, while in the large domains (e.g. 70 x 70 μm), cells were well-expand (flat shape) and Yap was in the nuclei (Fig. 2, middle and right). These results suggest that cell shape regulates Yap subcellular distribution independent of cell-cell contact or adhesion.

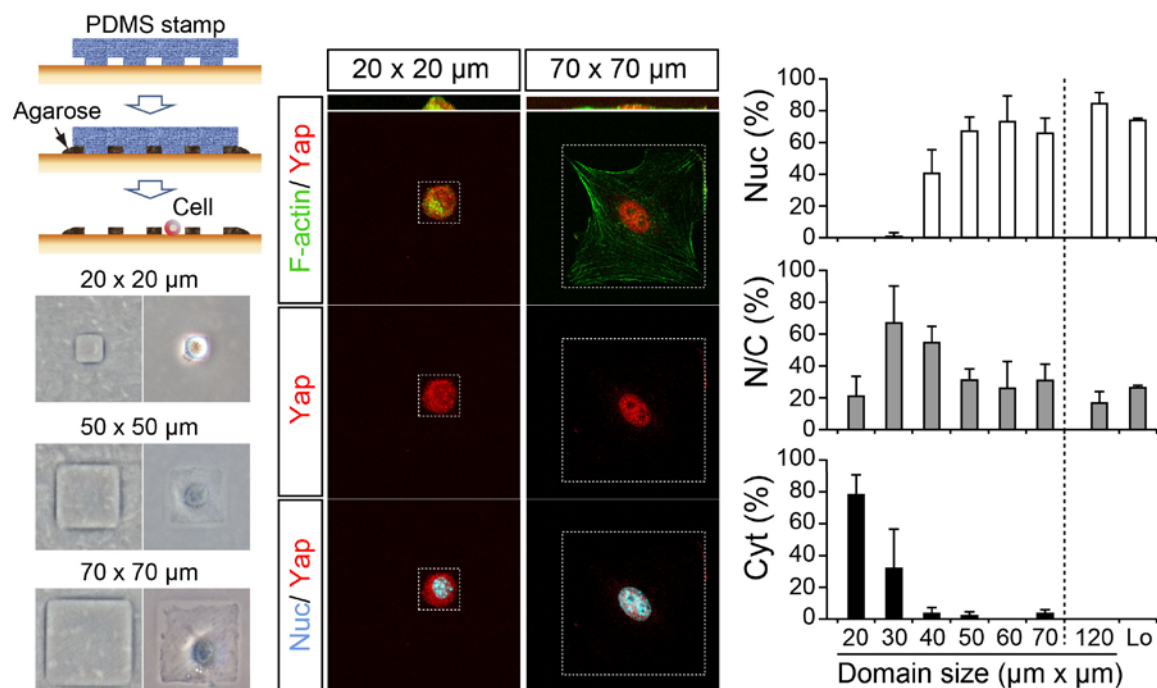


Fig. 2. Cell shape regulates Yap subcellular distribution independent of cell-cell contact. (Left) Schematic illustration of microdomain production and cells cultured on the microdomains. (Middle and right) Yap distribution pattern in the cells cultured on various-sized microdomains. Yap, nuclei (Nuc), and F-actin are indicated in red, cyan, and green, respectively. Yap distribution was examined 18 h after seeding on microdomains.

Since it is well documented that cytoskeleton proteins, including F-actin tightly relates with cell shape, we examined the effect of F-actin on Yap subcellular localization pattern. On 20 x 20 μm domains, stress fibers (thick fibrous structure of F-actin) were barely observed. However on 50 x 50 μm domains, stress fibers were clearly present (Fig. 3, left). Therefore, cells change the stress fiber quantity in response to cell shape. We also found that disruption of F-actin by Cytochalasin D (CytD) treatment reduced the nuclear localization of Yap even in

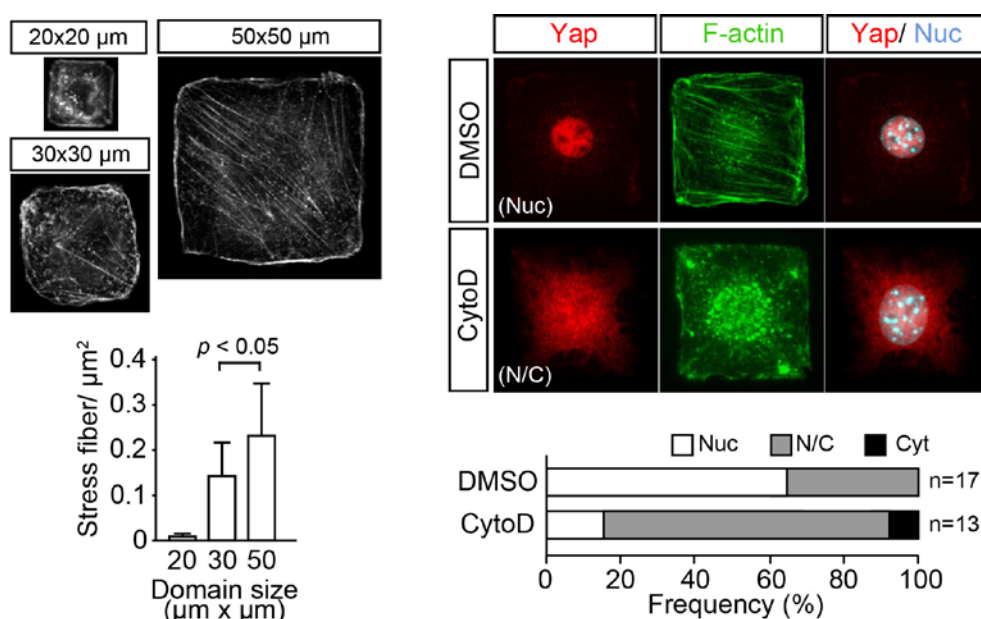


Fig. 3. Cell shape regulates Yap by modulation of F-actin/stress fibers. (Left) F-actin distribution in NIH3T3 cells cultured on various-sized microdomains. (Right) Yap distribution was compared among well-expand cells cultured on 50 x 50 μm domains with or without of cytochalasin D (CytD) treatment.

well-expand state (Fig. 3, right), suggesting that F-actin regulated Yap down stream of cell shape.

To examine whether F-actin/stress fibers function through Hippo signaling pathway, we manipulated the activity of Lats protein kinase by overexpression of Lats2 or kinase-defective form of Lats2 (Lats2-KD) that is dominant negative for Lats1/2. We found that in Lats2-KD-expressing cells, nuclear Yap was maintained following CytoD treatment (Fig. 4). Since Lats phosphorylates five serine residues including S112 in mouse Yap and inhibits nuclear

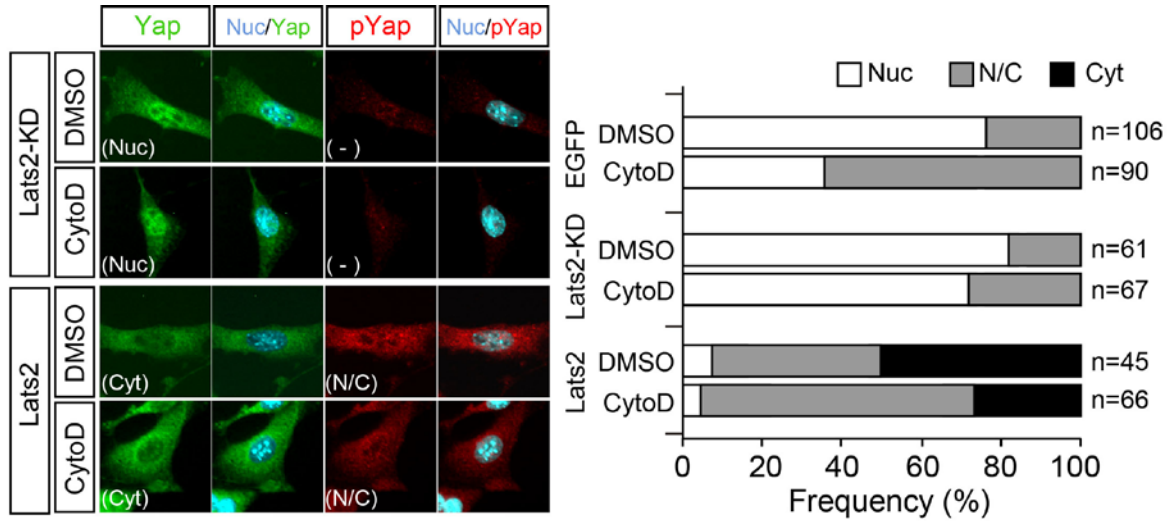


Fig. 4. F-actin regulates Yap through Hippo signaling pathway. EGFP (as a control), Lats2-KD, and Lats2 were overexpressed in NIH3T3 cells. To identify the transfectants, EGFP expression vector was co-transfected in each experiments. S112-phosphorylated Yap was detected by anti-phosphorylated Yap antibody (pYap).

localization of Yap, the most probable explanation of these results is that F-actin/stress fibers have an inhibitory effect on Hippo signaling pathway at or more upstream point(s) of Lats.

Based on these findings, we propose a novel model of 'cell contact inhibition of proliferation' (Fig. 5). At low cell densities, cells are well-expand (flat shape), and this shape promotes the formation of stress fibers. Stress fibers inhibit the Hippo pathway upstream or at Lats, thereby reducing Yap phosphorylation and promoting nuclear Yap accumulation. In the nucleus, Yap binds to the Tead family of transcription factors and promotes cell proliferation. By contrast, at high cell densities, cells become less-expand (round shape) and this shape reduces stress fibers and activates Hippo signaling, leading phosphorylation of Yap to exclude from the nucleus.

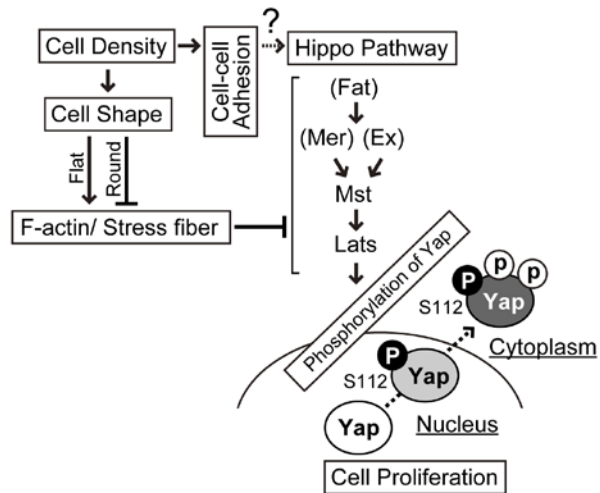


Fig. 5. A novel cell density-dependent model of 'cell contact inhibition of cell proliferation'.

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