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Hippo signaling pathway transcription co-activator YAP is localized to podosomes/invadopodia in Src-transformed NIH-3T3 fibroblasts

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Abstract
Many cancer cells or transformed fibroblasts make invadopodia, which are actin-rich adhesive organelles that are capable of remodeling the extracellular matrix. Invadopodia, and closely related podosomes, facilitate cell migration and invasion through the tissues. Recently, it was shown that Amotl2 plays a crucial role in invadopodia formation in Src-transformed fibroblasts by regulating actin cytoskeleton organization. One function of Amotl2 is regulation of the Hippo signaling pathway through the interaction with YAP transcription co-activator. Actin-associated Amotl2 sequesters cytoplasmic YAP and inhibits its nuclear translocation and transcription of Hippo-dependent genes. In this study, we investigated localization of YAP in Src-3T3 cells and observed that this protein concentrates at actin-rich invadopodia. This result suggests that YAP is a novel invadopodia component and may regulate organization of these organelles.

Introduction
The Hippo pathway plays an important role in many processes such as apoptosis, cell proliferation, differentiation, cell polarity and cancer progression [1] [2]. Components of the Hippo signaling cascade are recognized as tumor suppressors. The YAP (Yes-associated protein) is a downstream effector of the Hippo pathway and is negatively regulated by phosphorylation. When dephosphorylated, YAP translocates to the nucleus where it binds to transcription factor TEAD, which induces transcription of Hippo-controlled genes [3]. The nuclear translocation of YAP is regulated by the angiomotin family of proteins that localize to the actin cytoskeleton and sequester YAP in the cytoplasm which blocks transcription of Hippo regulated genes [4]. Recently, it has been shown that one of the angiomotin family members, Amotl2 (angiomotin-like-2), localizes to podosomes and invadopodia in various cell types, including Src-transformed NIH-3T3 fibroblasts, and regulates their organization [5] [6]. Here, we show that Amotl2-binding protein YAP is concentrated at the invadopodia in Src-3T3 cells.

Objective
The aim of this experiment is to determine if YAP is localized to invadopodia in Src-transformed fibroblasts.

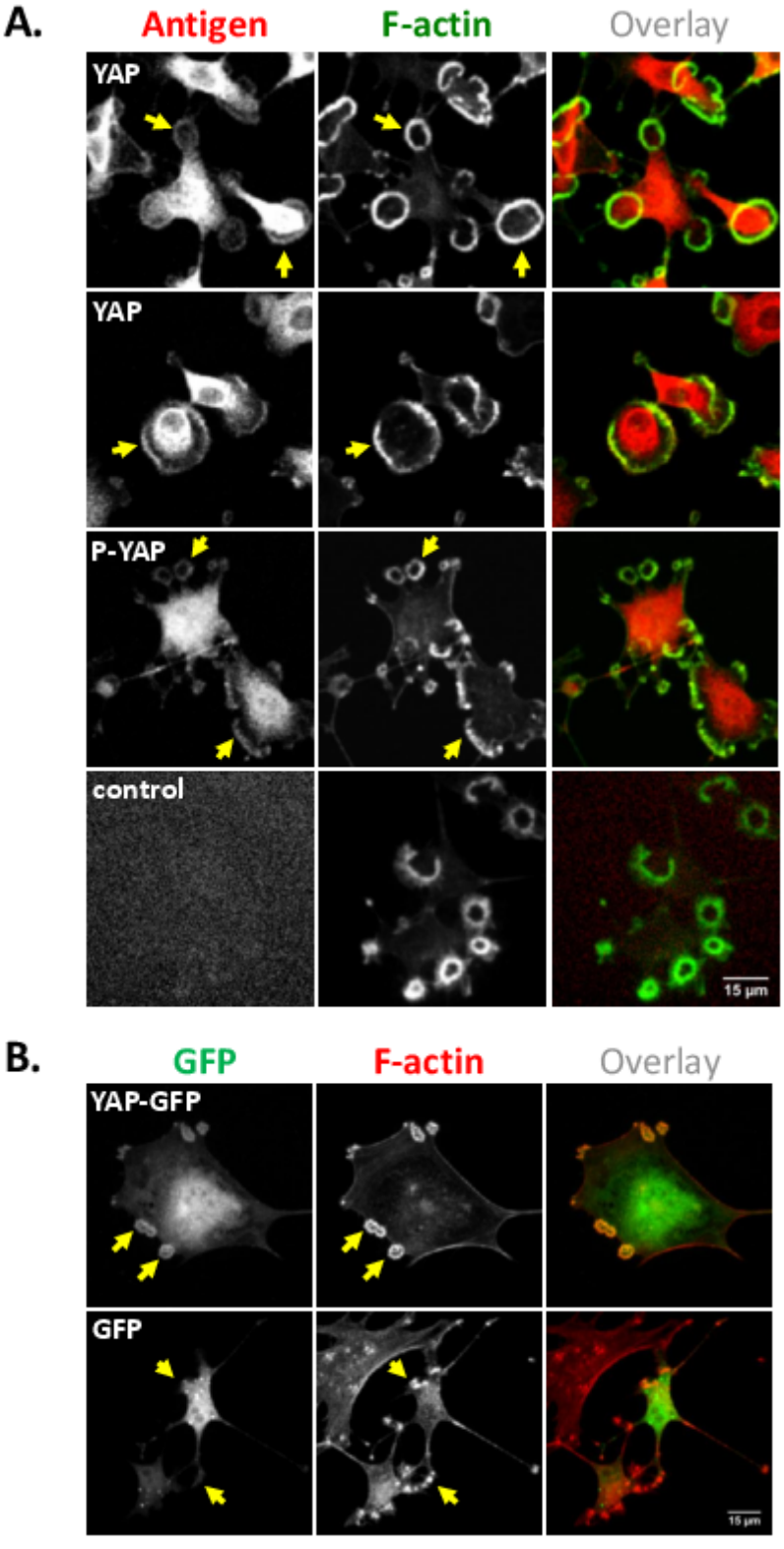

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Figure Legend

Figure 1.

(A) YAP localization to invadopodia in Src-3T3 cells. Cells were stained with Phalloidin-Alexa-488 (green) to visualize actin-rich invadopodia and indicated antibodies (red) to visualize YAP. Two different examples of YAP staining are shown. In the control exper-

iment we omitted primary antibody in the staining procedure and enhanced contrast to demonstrate that no signal was detected. Arrows point to invadopodia. Scale bar 15 µm.

(B) YAP-GFP localization to invadopodia in Src-3T3 cells. Cells were transfected with YAP-GFP fusion construct or GFP alone (control), fixed and stained with Phalloidin-Alexa-568 (red) to visualize actin-rich invadopodia. YAP-GFP fluorescence (green) was detected at invadopodia. Arrows point to invadopodia. Scale bar 15 µm.

Cell culture

Src-transformed NIH-3T3 cells (a generous gift from Sara Courtneidge, Sanford-Burnham Medical Research Institute, La Jolla, USA) [10] were cultured in DMEM (Dulbecco's modified Eagle's medium, Lonza) containing 10% FBS (Eurx) and 1% penicillin/streptomycin (Life Technologies). For experiments, cells were plated in 24 well plates on glass coverslips.

Transfection and staining

Cells were transfected with pEGFP-C3-hYAP1 (Addgene) [11] using Lipofectamine 2000 (Invitrogen), according to the manufacturer instructions, and fixed with 3% paraformaldehyde and 0.1% glutaraldehyde 48 h after transfection. To quench glutaraldehyde, cells were incubated with 0.2% sodium borohydride and washed with PBS. After permeabilization with 0.5% Triton X-100 for 30 min, F-actin was visualized with Acti-stain-555 Phalloidin (Cytoskeleton).

Immunostaining

Cells were fixed with 3% paraformaldehyde or acetone and blocked for 1 h with 2% BSA (Bovine Serum Albumin, Sigma), 2% NGS (Normal Goat Serum, Jackson Laboratory) in the presence of 0.5% Triton X-100. After blocking, cells were incubated overnight with rabbit anti-YAP (Aviva) or rabbit anti-P-YAP (Genetex) antibodies. Alexa-568 conjugated secondary antibody (Invitrogen) was used to visualize the antigen and Acti-stain-488 (Cytoskeleton) to stain F-actin.

Microscopy

Images were obtained using Spinning Disc microscope with 40x/1.20 water objective (Zeiss) and were analyzed with ImageJ (NIH) software.

Results & Discussion

To determine YAP localization in Src-3T3 cells, we performed immunocytochemistry using anti-YAP and anti-phospho-YAP (P-YAP) antibodies and in order to visualize invadopodia, we counterstained cells with phalloidin that labels F-actin. We observed significant immunoreactivity at invadopodia using anti-YAP antibody (Fig. 1A). To verify the specificity of anti-YAP staining we performed staining with another antibody against YAP. The second antibody available to us recognizes YAP specifically in its phosphorylated state (P-YAP). Consistent with the previous result, P-YAP immunoreactivity was enhanced at the invadopodia (Fig. 1A). No immunofluorescence was observed in control staining omitting primary antibody, even after enhancing the contrast (Fig. 1A). To confirm our immunocytochemical observations, we transfected Src-3T3 cells with plasmids expressing YAP-GFP fusion protein. In control experiments, cells were transfected with soluble GFP. In contrast to GFP, YAP fusion construct was strongly enriched at invadopodia (Fig. 1B). Collectively, our experiments suggest that YAP is a novel invadopodia-associated protein.

We envisioned that YAP could be localized to invadopodia for two reasons. Firstly, YAP is known as an interacting protein to all angiomotins [4]. Secondly, in MDCK (Madin-Darby canine kidney) cells, Amotl2 was shown to localize to the tight junction and recruit YAP [7] [4]. Invadopodia in Src-3T3 cells, similar to tight junctions in epithelial cells, are sites of cellular adhesion enriched in actin and Amotl2. It will be interesting to know if YAP also has a functional role in the organization of invadopodia and podosomes. If yes, it would be important to check if other components of the Hippo signaling pathway are also involved in the formation of invadopodia. This could constitute another mechanism through which the Hippo signaling pathway impinges on cancer cell organization and metastasis [8] [9].

Conclusions

YAP is localized to invadopodia in Src-3T3 cells.

Additional Information

Methods

Cell culture

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Supplementary Material

Please see https://sciencematters.io/articles/201602000035.

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Ethics Statement

The work presented here does not trigger ethical concerns.

Citations

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