HRVATSKO MIKROSKOPIJSKO DRUŠTVO

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u organizaciji Suzane Šegota
uz sljedeći

D n e v n i r e d:

1. **Predavanje stipendista 4. hrvatskog mikroskopijskog kongresa:**
   - **Lucija Mijanović:** IqgC at the crossroads of RasGAP and IQGAP protein families
   - **Krunoslav Vinicki:** Fully automatic whole slide imaging using faster than real-time image recognition neural network

2. **Razno**

Tajnica:
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IqgC at the crossroads of RasGAP and IQGAP protein families

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Ras-specific GTPase activating proteins (RasGAPs) constitute a diverse group of proteins characterized by their GAP domain. This domain binds and inactivates small GTPases from the Ras family by stimulating their GTP hydrolytic activity. Despite having a highly homologous GAP-related domain (GRD), members of the IQ-motif containing Ras GTPase-activating-like protein (IQGAP) family do not function as RasGAPs. They are multidomain proteins that serve as scaffolds for various pathways and modulate diverse cellular processes [1]. Amoeba *Dictyostelium discoideum* encodes four IQGAP-related proteins – DGAP1, GAPA, IqgC and IqgD. DGAP1 and GAPA are extensively studied and exhibit traditional IQGAP activity, i.e. they participate in the formation of large protein complexes involved in the regulation of actin cytoskeleton and are unable to inactivate Ras GTPases. We showed recently that IqgC, despite apparently belonging to the IQGAP family, is a genuine RasGAP [2]. It binds and inactivates small GTPase RasG, acting as a negative regulator of large-scale endocytosis. Based on the observation that *iqgC*-null cells detach easily from the cell culture dishes, we set out to characterize the role of IqgC in the cell-substratum adhesion. Shaking assays showed that *iqgC*-null cells adhere considerably more weakly to the glass surface than the wild-type cells, and expression of recombinant IqgC in mutant cells rescued this phenotype. IqgC localizes to the punctate adhesion structures together with the adhesion marker paxillin B, as shown by total internal reflection fluorescence (TIRF) microscopy. We expressed fluorescently labeled IqgC in *rasG*-null cells and examined them using confocal microscopy. Normal localization of IqgC to the adhesion foci was observed, indicating that RasG is not necessary for its role in adhesion. To further dissect the role of IqgC in the cell-substratum adhesion, we expressed its individual fluorescently labeled domains in wild-type cells and examined their localization: the GRD domain with GAP activity towards RasG, and the RGCt domain unique to IQGAPs. YFP-RGCt, but not YFP-GRD, localized to the adhesion foci. The same constructs were tested for their ability to rescue the adhesion phenotype of *iqgC*-null cells in shaking assays, but neither could completely rescue the defect. We conclude that the interaction of IqgC with RasG and its GAP activity is not essential for the role of IqgC in the cell-substratum adhesion. The RGCt domain of IqgC is necessary and sufficient for the proper localization of the protein to the adhesion foci, but the expression of the full-length protein is necessary to restore normal adhesion. Since the RGCt domain is a hallmark of IQGAPs, our results suggest that IqgC unifies the features and activities of both RasGAP and IQGAP protein families in the regulation of various cellular processes.

Keywords: IqgC, *Dictyostelium discoideum*, adhesion, GAP, IQGAP

References:
1. J.M. Smith et al., Trends. Cell. Biol. 25 (2015) 171-184.
2. M. Marinović et al., PNAS USA. 116 (2019) 1289-1298.
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Fully automatic whole slide imaging using a faster than real-time image recognition neural network

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In pathology, digitization of glass slides with specimens utilizing whole slide imaging (WSI) opens the door for the use of computer algorithms in image analysis, greatly increasing diagnostic objectivity and productivity [1]. But, despite its numerous advantages, the WSI still faces many technical challenges, especially at high numerical apertures where field of view (FOV) and depth of field (DOF) can be significantly reduced. Under these conditions, artifacts such as tissue folds, ink, dust, and air bubbles can limit the performance of tissue slide scanners, leading to digital slides with out-of-focus areas or even completely failed scans. In modern slide scanners, this problem is solved with different scanning modes, allowing users to choose between automated and semi-automated modes in which area of interest and even focus points can be manually set. In order to make microscope slide scanners more robust to various artifacts (Fig. 1) and enable "one-button" or fully automated scanning, we developed a faster than real-time, 500 fps neural network that can be deployed during the scanning process to decide, depending on what is in the current FOV, should the autofocus and stitching algorithms be engaged for those tiles and in what way. After training our neural network on 64 000 images separated into seven different classification categories, we obtained the accuracy of 97 %, effectively attaining the performance of a human operator.

Keywords: pathology, whole slide imaging, deep learning

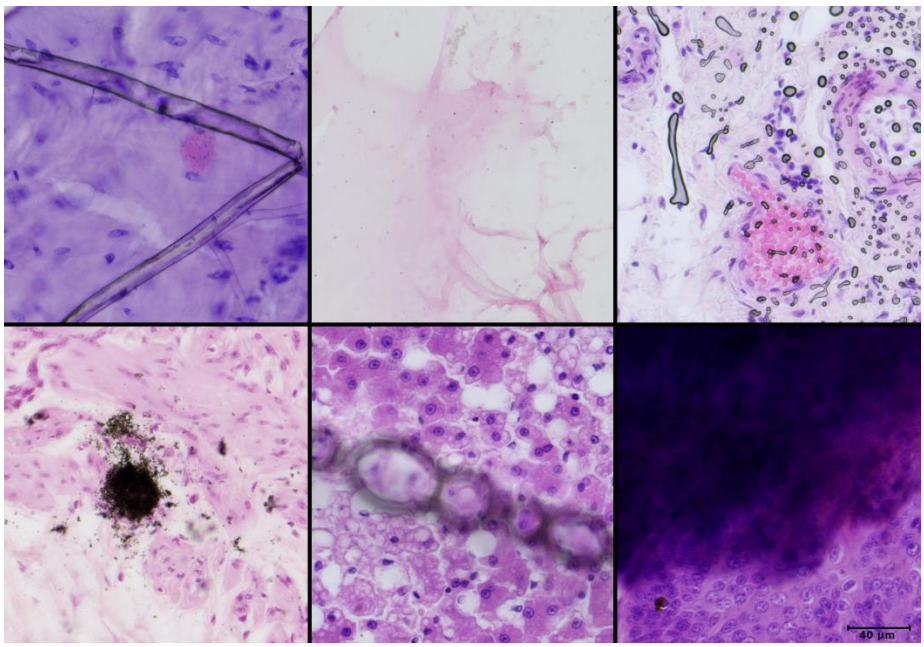

Figure 1. Regions of interest (ROIs) extracted from WSI showing various artifacts.

References:
1. F. Ghaznavi et al., Annu. Rev. Pathol. 8 (2013) 331–359.