Super Resolution Microscopy Identification of Focal Adhesion-Associated Caveolin-1 Domains

Timothy H. Wong *1, Mona Shahsavari*2, Ismail M. Khater2, Bharat Joshi1, Ghassan Hamarneh2, and Ivan Robert Nabi1&1

1Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, BC V6T 1Z3, Canada
2Medical Image Analysis Lab, School of Computing Science, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

July 2019

* & These authors contributed equally to this work.

Caveolin-1 (Cav1) is the coat protein for cell surface caveolae and is also localized to functional non-caveolar domains, or Cav1 scaffolds, that can be defined by network analysis of single molecule localization microscopy (SMLM) (Khater et al, Sci Rep, 2018). Cav1 is also a Src kinase substrate phosphorylated on tyrosine-14 (Y14). pCav1 regulates focal adhesion (FA) tension in prostate cancer cells using vinculin FRET tension sensor (Meng et al, MBC, 2017). CRISPR/Cas9-mediated knockout of Cav1 in a highly migratory MDA-MB-231 breast carcinoma clone decreases vinculin tension that is rescued by wild-type Cav1 and phosphomimetic Cav1Y14D but not non-phosphorylatable Cav1Y14F. We applied SMLM network analysis and machine learning based 3D pattern analysis tools to cells dually labeled for Cav1 and vinculin to analyze the features and types of Cav1 domains in proximity to FAs. We observed no difference in the distribution of caveolae and scaffolds with respect to FA proximity but did find that Cav1 domains close to FAs are denser and smaller. Interestingly, release of pCav1-dependent FA tension by either actin depolymerization with latrunculin A or Src inhibition with PP2 increased the number of Cav1 domains in proximity to FAs. Ongoing work will determine the role of Y14 phosphorylation on Cav1 domain distribution and characteristics in relation to FAs.

Supported by CIHR (PJT-159845, PJT-156424) and NSERC Discovery grants (IRN, GH)
Super Resolution Microscopy Identification of Focal Adhesion-Associated Caveolin-1 Domains

Timothy H. Wong\textsuperscript{1}, Mona Shahevari\textsuperscript{2}, Ismail M. Khater\textsuperscript{2}, Bharat Joshi\textsuperscript{1}, Ghassan Hamarnah\textsuperscript{3,4} & Ivan Robert Nabi\textsuperscript{1,5}

\textsuperscript{1} Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, BC V6T 1Z3, Canada.
\textsuperscript{2} Medical Image Analysis Lab, School of Computing Science, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
\textsuperscript{3} These authors contributed equally to this work.

1. Clones of parental MDA-MB-231 cells show varying cell morphology. Cav1 and Galectin-3 (Ga13) knockdown reduce vinculin tension in focal adhesions and cell migration

2. Cav1 CRISPR/Cas KO of the MDA-MB-231-2F10 clone. Cav1WT and Y14D, but not Y14F or CSD rescue vinculin tension

3. Single Molecule Localization Microscopy (SMLM) dSTORM imaging of 3D Cav1 and 2D vinculin focal adhesions

4. Network analysis and machine learning of SMLM data sets identify the molecular structure of Cav1 caveolae and non-caveolar scaffold domains

5. Proximity analysis of SMLM datasets: Correlating changes in features of caveolae and scaffold domains with distance to FAs

6. Reducing FA tension and actin polymerization increases the number of Cav1 domains in proximity to FAs. Domains closer to low tension FAs are less dense and more isotropic in shape

References