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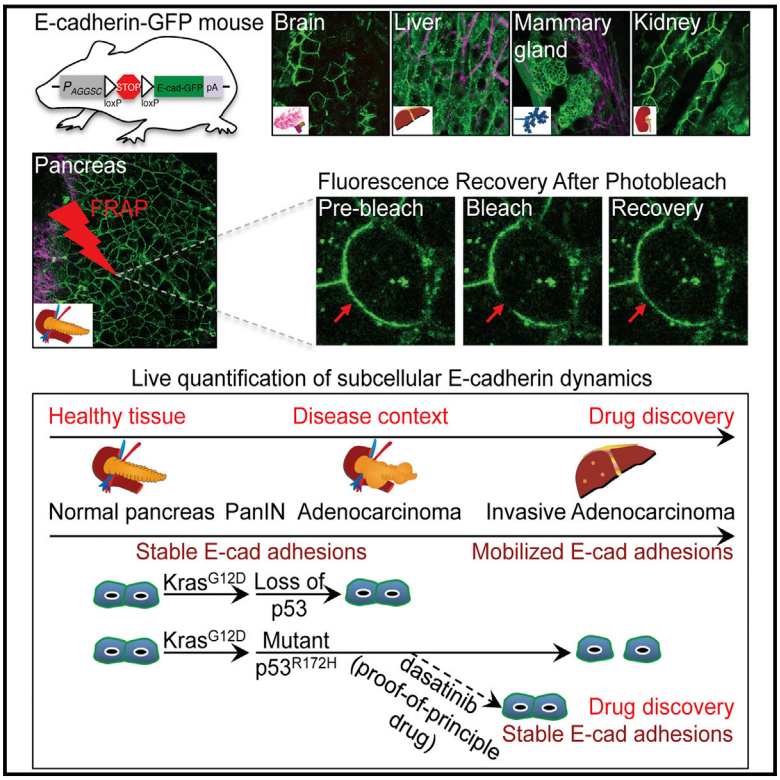
Intravital FRAP Imaging using an E-cadherin-GFP Mouse Reveals Disease- and Drug-Dependent Dynamic Regulation of Cell-Cell Junctions in Live Tissue

Graphical Abstract



Highlights

- The E-cadherin-GFP mouse allows in situ quantification of E-cadherin mobility
- We monitored E-cadherin mobility during tissue homeostasis and disease development
- Invasive pancreatic cancer driven by mutant Kras/p53 increases E-cadherin mobility
- Dasatinib treatment reverts E-cadherin mobility and reinforces tumor cell junctions

Authors

Zahra Erami, David Herrmann, Sean C. Warren, ..., Jennifer P. Morton, Kurt I. Anderson, Paul Timpson

Correspondence

k.anderson@beatson.gla.ac.uk (K.I.A.), p.timpson@garvan.org.au (P.T.)

In Brief

Erami et al. generate an E-cadherin-GFP mouse to demonstrate real-time quantification of E-cadherin mobility using intravital photobleaching in a range of tissue types. They show that changes in E-cadherin mobility correlate with changes in cell junction integrity and invasiveness while demonstrating applications of the mouse for future drug discovery studies.

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Intravital FRAP Imaging using an E-cadherin-GFP Mouse Reveals Disease- and Drug-Dependent Dynamic Regulation of Cell-Cell Junctions in Live Tissue

Zahra Erami,[1,5] David Herrmann,[2,5] Sean C. Warren,[2] Max Nobis,[1] Ewan J. McGhee,[1] Morghan C. Lucas,[2] Wilfred Leung,[2] Nadine Reischmann,[2] Agata Mrowinska,[1] Juliane P. Schwarz,[1] Shereen Kadir,[1] James R.W. Conway,[2] Claire Vennin,[2] Saadia A. Karim,[1] Andrew D. Campbell,[1] David Gallego-Ortega,[2] Astrid Magenau,[2] Kendelle J. Murphy,[2] Rachel A. Ridgway,[1] Andrew M. Law,[2] Stacey N. Walters,[2] Shane T. Grey,[2] David R. Croucher,[2] Lei Zhang,[2] Herbert Herzog,[2] Edna C. Hardeman,[3] Peter W. Gunning,[4] Christopher J. Ormandy,[2] T.R. Jeffry Evans,[1] Douglas Strathdee,[1] Owen J. Sansom,[1] Jennifer P. Morton,[1] Kurt I. Anderson,[1,6,*] and Paul Timpson[2,6,*]

[1]Cancer Research UK Beatson Institute, Switchback Road, Bearsden, Glasgow G61 1BD, UK

[2]The Garvan Institute of Medical Research and The Kinghorn Cancer Centre, Cancer Division, St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney, NSW 2010, Australia

[3]Neuromuscular and Regenerative Medicine Unit, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia

[4]Oncology Research Unit, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia

[5]Co-first author

[6]Co-senior author

*Correspondence: k.anderson@beatson.gla.ac.uk (K.I.A.), p.timpson@garvan.org.au (P.T.)

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SUMMARY

E-cadherin-mediated cell-cell junctions play a prominent role in maintaining the epithelial architecture. The disruption or deregulation of these adhesions in cancer can lead to the collapse of tumor epithelia that precedes invasion and subsequent metastasis. Here we generated an E-cadherin-GFP mouse that enables intravital photobleaching and quantification of E-cadherin mobility in live tissue without affecting normal biology. We demonstrate the broad applications of this mouse by examining E-cadherin regulation in multiple tissues, including mammary, brain, liver, and kidney tissue, while specifically monitoring E-cadherin mobility during disease progression in the pancreas. We assess E-cadherin stability in native pancreatic tissue upon genetic manipulation involving Kras and p53 or in response to anti-invasive drug treatment and gain insights into the dynamic remodeling of E-cadherin during in situ cancer progression. FRAP in the E-cadherin-GFP mouse, therefore, promises to be a valuable tool to fundamentally expand our understanding of E-cadherin-mediated events in native microenvironments.

INTRODUCTION

The capacity of cancer cells to dissociate from primary tumors and invade requires the deregulation of interactions with adjacent cells and the surrounding tissue. A major challenge in biology is the real-time monitoring of protein dynamics involved in this process in their native context (Conway et al., 2014). The ability to quantify the intricate spatiotemporal regulation of cell adhesion molecules, such as E-cadherin, using techniques including fluorescence recovery after photobleaching (FRAP) has rapidly enhanced our understanding of E-cadherin's subcellular roles in regulating cell-cell integrity and dissociation in vitro (Canel et al., 2010a; Shen et al., 2008; Sprague and McNally, 2005; Wu et al., 2014).

FRAP is commonly used for monitoring molecular movement within cells. A small fluorescent region is bleached, and fluorescence recovery into the bleached region is measured over time (Axelrod et al., 1976; Fritzsche and Charras, 2015; Sprague and McNally, 2005). From this, multiple readouts can be derived, including, but not limited to the half-time of recovery, a measure of the rate at which fluorescent molecules move in or out of the region of interest, and the immobile fraction, an indication of how much of the molecule remains trapped and unable to move out of the analyzed region (for in-depth insights into FRAP analysis, see Fritzsche and Charras, 2015). In the case of fluorescently labeled E-cadherin, the immobile fraction can indicate how much E-cadherin is trapped or engaged in cell-cell junctions and may provide a molecular readout of junction stability in real time (Canel et al., 2010b; Serrels et al., 2009).

FRAP has largely been used to probe molecular events within 2D cell culture using transfection-based approaches (Lippincott-Schwartz et al., 2001; Shen et al., 2008), whereas its utility in vivo has been limited (Ellenbroek and van Rheenen, 2014). Recently, we and others have used FRAP in more complex and physiologically relevant environments ranging from application in *Drosophila* (Cavey et al., 2008) to the use of E-cadherin-GFP FRAP in a mammalian system in vivo (Serrels