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### Publications

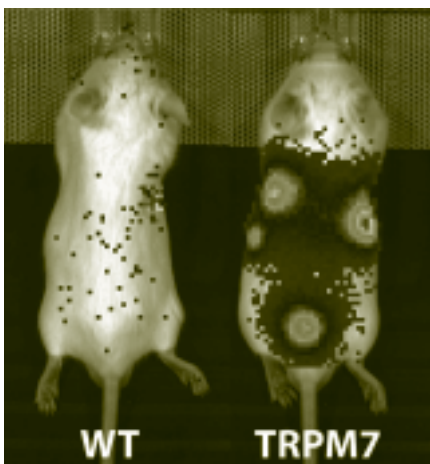
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Goedhart J, van Weeren L, Hink MA, Vischer NO, Jalink K, Gadella TW, Jr. *Bright cyan fluorescent protein variants identified by fluorescence lifetime screening.* *Nat Methods* 2010;7:137-9

Jalink K, Moolenaar WH. *G protein-coupled receptors: the inside story.* *Bioessays* 2010;32:13-6

Borst JW, Willemse M, Slijkhuis R, van der Krogt G, Laptinok SP, Jalink K, Wieringa B, Fransen JA. *ATP Changes the Fluorescence Lifetime of Cyan Fluorescent Protein via an Interaction with His148.* *PLoS ONE* 2010;5:e13862

Gloerich M, Ponsioen B, Vliem MJ, Zhang Z, Zhao J, Kooistra MR, Price LS, Ritsma L, Zwartkruis FJ, Rehmann H, Jalink K, Bos JL. *Spatial regulation of cyclic AMP-Epac1 signaling in cell adhesion by ERM proteins.* *Mol Cell Biol* 2010;30:5421-31



## BIOPHYSICS OF CELL SIGNALING

Employing advanced imaging and other biophysical techniques, we study cell signaling events with high spatial and temporal resolution. Electrophysiological (e.g. patch clamping) and advanced imaging (e.g. Fluorescence Resonance Energy Transfer (FRET), Fluorescence Lifetime Imaging (FLIM), Fluorescence Cross Correlation Spectroscopy (FCCS) and photorelease of caged compounds) are used in research projects in our group as well as in a number of collaborations within and outside our institute. We also contribute to the development of hardware, software and FRET sensors for various intracellular messengers.

**The cation channel TRPM7 in the control of invadosomes and invasive migration.** Podosomes and invadopodia are related cytoskeletal structures implicated in (tumor) cell invasion. These “invadosomes” mediate cell-matrix contact, sense mechanical forces and serve as focal secretion sites for proteases that degrade the extracellular matrix. Invadosomes consist of an actin-dense core surrounded by a characteristic juxtamembrane ring containing contractile and regulatory proteins. Among these are force-generating myosins, actin-bundling and -capping proteins, signaling proteins and proteins involved in secretion of proteases. In an ongoing collaboration with the group of Dr. F.N. van Leeuwen (Nijmegen) we identified the atypical ‘channel-kinase’ TRPM7 as novel component of the invadosome ring. TRPM7 is a membrane ion channel fused to a protein kinase domain which functions as a mechanosensor and regulator of local  $Ca^{2+}$  entry. Strikingly, forced expression of TRPM7 in neuroblastoma cells is sufficient to induce invadosome formation. Phospholipase C (PLC) signaling triggers TRPM7-mediated  $Ca^{2+}$  influx and enhances invadosome formation. Thus, TRPM7 may function as a master regulator of invadosomes under the control of GPCR signals.

We found that TRPM7 confers a highly invasive phenotype on otherwise non-invasive neuroblastoma cells, both in vitro (time-lapse imaging, Transwell assays) and in vivo (tail-vein injection in nude mice). Conversely, RNAi-mediated knockdown of TRPM7 strongly suppresses migration in MDA-MB-231 breast carcinoma cells, a model for invasive breast cancer. Moreover, mRNA expression profiling of 246 human breast carcinoma specimens reveals that high expression of TRPM7 at the time of diagnosis predicts a poor prognosis and is correlated with distant metastasis. This result has now been confirmed in large published databases and, by QPCR, in an independent set of tumor biopsies in Nijmegen. These findings provide strong support for a role of TRPM7 in tumor cell dissemination. Mass spectrometry analysis of TRPM7 immuno-complexes identified some 40 proteins implicated in cytoskeletal regulation, cell adhesion and -migration. The large majority of these proteins localizes to invadosomes. Proteins associated with  $Ca^{2+}$ /PLC signaling are amply represented in the set, suggesting an important role for these intracellular messengers. Consistent with this notion we find that TRPM7 mediates local  $Ca^{2+}$  influx to specifically activate PLC $\delta_1$  in invadosomes, leading to PIP<sub>2</sub> hydrolysis and sustaining the TRPM7 open-channel state. Our current investigations address, by combining biophysical readout techniques with mutational analysis of the TRPM7 C terminus, the details of TRPM7 sensitivity to  $Ca^{2+}$  and phosphoinositides, and the exact role of PLC $\delta_1$  which appears to mediate a  $Ca^{2+}$ -influx dependent feedback loop in the activation of TRPM7. We have also started analyzing localization and role of novel invadosome components identified in the mass spec screen. Strikingly, a high percentage of the protein components identified in the mass spectrometry screen also correlate significantly with poor outcome.

Figure 5: TRPM7 overexpression significantly enhances neuroblastoma metastasis to liver. Tail vein-injected N1E-115 neuroblastoma cells specifically metastasize to liver and bone. Neuroblastomas that overexpress TRPM7 just ~3-fold over endogenous levels are much more effective in invading those tissues. Tumor load was visualized by luciferase imaging.