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## GENETIC CONTROL OF INVASION AND METASTASIS

The aim of our research is to identify and characterize genes that play an essential role in processes of tumor formation and in particular tumor progression. Insight into the molecular mechanisms that underlie the progression of tumors may lead to the development of novel diagnostic tools or improved therapeutic strategies.

**Rho family proteins** Using functional screens we have identified different invasion-inducing genes. Of these, the invasion-inducing *Tiam1* gene encodes an activator (GEF) for the Rho-like GTPase Rac. Rho family proteins, which include Cdc42, Rac1 and RhoA, control a wide range of biological processes such as cell adhesion, cell migration and cell polarity. In particular, they act in signaling pathways that regulate the reorganization of the actin cytoskeleton in response to receptor stimulation.

**Tiam1-Rac signaling and cell polarity** Complex formation of GEFs with various scaffold proteins is an important mechanism to determine signaling towards and downstream of Rho GTPases. *Tiam1* associates with Par3 of the Par polarity complex - consisting of Par3, Par6 and PKC $\zeta$  - and thereby regulates polarity processes in various cell types and in different contexts. *Tiam1* promotes E-cadherin-based cell-cell adhesions in epithelial cells whereas knock down of *Tiam1* leads to loss of cell-cell adhesions and epithelial-mesenchymal transition in MDCKII cells. In keratinocytes, we found that *Tiam1* controls tight junction (TJ) formation by activation of the Par polarity complex, which is required for the establishment of apical-basal epithelial cell polarity. Interestingly, both Par3 and *Tiam1* bind independently to the junctional adhesion molecule A (JAM-A), which functions as a membrane localized scaffold to initiate epithelial cell polarity. JAM-A orchestrates the link between the Par-complex and *Tiam1*-mediated Rac signaling to control cell polarization.

In the absence of cell-cell adhesions, epithelial cells develop front-rear polarization and migrate in a persistent fashion involving *Tiam1*-Par complex signaling. Upon the induction of migratory signalling cascades in astrocytes, the MTOC and the Golgi apparatus are reoriented in a position in front of the nucleus, a process dependent on Cdc42. The MTOC faces the direction of migration, and *Tiam1* is required for the Rac-mediated protrusion outgrowth in astrocytes but not for the Cdc42-mediated MTOC orientation. In T lymphocytes, *Tiam1*-Par signaling is required for chemokine- and S1P-induced Rac activation and subsequent directional cell migration. *Tiam1*-deficient T-cells show reduced chemotaxis *in vitro*, and impaired homing, egress and contact hypersensitivity *in vivo*. Par/PKC $\zeta$ /*Tiam1*/Rac signaling is essential for the stabilization of polarization and efficient crawling of T-cells on endothelial cells.

**Tumorigenicity in *Tiam1*-deficient mice** *Tiam1*-deficient (*Tiam1*<sup>-/-</sup>) mice develop, grow, and reproduce normally. In mouse skin, *Tiam1* is present in basal and suprabasal keratinocytes of the epidermis as well as in hair follicles. *Tiam1*<sup>-/-</sup> mice display resistance to DMBA/TPA-induced (Ras-induced) skin tumor formation but the frequency of malignant conversion of the tumors that do develop is increased. We also studied the function of *Tiam1* in tumorigenesis induced by other oncogenic signaling pathways in different cell types. *Tiam1* is a Wnt-responsive gene and promotes b-catenin/TCF-induced intestinal tumor formation. In addition, mammary tumor formation and progression induced by MMTV-c-neu - but not MMTV-myc - are delayed and reduced in the absence of *Tiam1*. In general, during tumor initiation *Tiam1*-mediated Rac activation promotes survival signals and thereby prevents apoptosis leading to increased tumorigenicity. As *Tiam1* often functions in conjunction with the Par polarity complex and polarity proteins may play a role in processes related to different aspects of cancer, we are currently analyzing the function of polarity proteins in tumor formation. Our results indicate that similarly to *Tiam1*-deficiency, deletion of Par3 in mouse leads to a reduced number of DMBA/TPA induced skin tumors. Par3-deficient keratinocytes show also lower survival signals and increased apoptosis *in vitro*, suggesting that *Tiam1* and Par polarity proteins affect tumorigenicity by acting in the same signaling pathway.

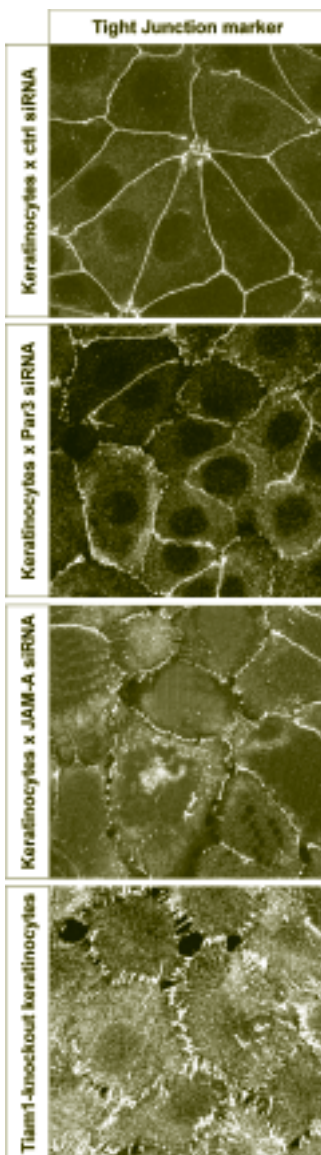


Figure 3: Keratinocytes that lack either the Par3 polarity protein, the junctional adhesion molecule JAM-A or the Rac activator *Tiam1*, show impaired tight junction maturation compared to control keratinocytes (siCtrl). As Par3, *Tiam1* and JAM-A associate with each other these data suggest that these proteins act in concert to establish epithelial cell polarity.

## ACTIN DYNAMICS IN CANCER CELLS

The polymerization of actin monomers into filaments produces mechanical force that sculpts protrusions and invaginations on membranes. Actin dynamics control the remodeling of the plasma membrane and are essential to support cell migration. Not surprisingly, sophisticated mechanisms have evolved to harness the activity of actin nucleators, enzymes required for actin to efficiently form filaments. To date, the Arp2/3 complex and Formins are the best characterized actin nucleators in mammalian cells.

Actin polymerization by the Arp2/3 complex results in the formation of new actin filaments arranged in a dendritic meshwork. The WASP/WAVE family of Nucleation-Promoting Factors (NPFs) stimulates the weak basal activity of the Arp2/3 complex. WAVE proteins are involved in the formation of lamellipodia, veil-like protrusion of the plasma membrane. Lamellipodia/ruffles are the primary organelles of motility for cells adopting mesenchymal-type movement. WAVE proteins confine Arp2/3-complex activity to the lamellipodium tip, which faces the plasma membrane. In this way, localized actin polymerization allows the plasma membrane enclosing lamellipodia/ruffles to advance. WASP proteins are instead implicated in endocytosis and trafficking: they activate the Arp2/3 complex on clathrin-coated pits and cytosolic vesicles.

Actin polymerization by the Diaphanous-related Formin mDia2 controls the formation of filopodia, finger-like extensions of the plasma membrane. At variance from lamellipodia, the role of filopodia in cell migration is still debated. mDia2 also regulates vesicle trafficking, which provides supplies to the leading edge of crawling cells.

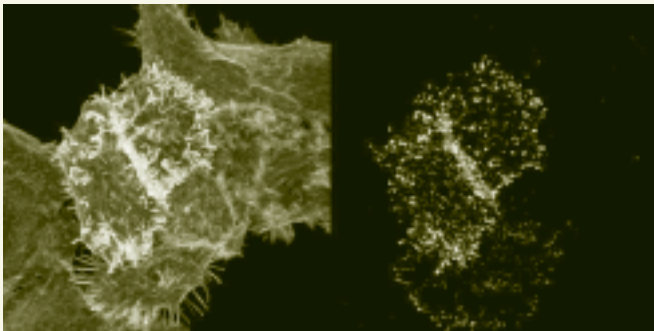


Figure 4: HeLa cells transfected with a constitutively active mutant of mDia2. Filamentous actin (left) and mDia2 (right) are displayed.

**Regulation of WAVE2- and N-WASP-based complexes** WAVE- and N-WASP-based core complexes have been shown to spatially and temporally restrict Arp2/3-dependent actin polymerization. However, they fail to provide a mechanistic explanation for the high versatility of the WASP/WAVE proteins.

Our research is revealing that dedicated subunits are required to confer functional specificity on both WAVE- and N-WASP-based core complexes. Moreover, these studies are uncovering new and NPF-independent roles for these proteins and unexpected links between different classes of actin nucleators.

**Regulation of mDia2** How mDia2 regulates actin dynamics is still matter of debate. In order to fully understand the biological function(s) of mDia2, we have isolated its interactome. This information is helping us dissect the mechanics controlling actin nucleation by mDia2. In addition, it is suggesting new roles for mDia2.

**Mechanisms of formation and roles of filopodia in cell migration** Although filopodia are likely to be fundamental in completing both developmental and homeostatic programs, little is known about their formation and functions in cell migration. Do filopodia originate from lamellipodia and steer crawling cells or are they repressed by lamellipodia and counteracting cell locomotion? To answer these questions, we have recently generated a genetically modified cell line that allows us to induce filopodium formation *in vitro* and to perform systematic studies. This tool enables us to undertake pioneering loss-of-function genetic and proteomic screens that will inventory filopodium-regulatory proteins and reveal the filopodium protein signature, respectively.



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#### Publications

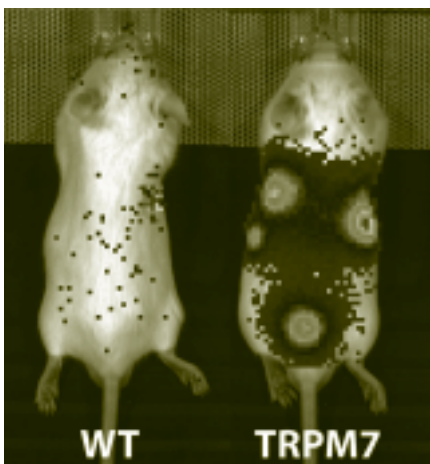
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## BIOPHYSICS OF CELL SIGNALING

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.