

The Action of Small GTPases Rab11 and Rab25 in Vesicle Trafficking During Cell Migration

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Key Words

Rab GTPases • Vesicular Trafficking • Recycling • Cancer cell migration

Abstract

Background: The closely related GTPases Rab11 and Rab25 promote cell migration by regulating vesicular transport and recycling of surface receptors. Rab25 carries a constitutively activating mutation in its GTPase domain. Increased expression of Rab25 has been associated with the aggressiveness of migrating tumor cells. Here, we aimed to elucidate potential differences in the role of those two GTPases in vesicle trafficking during cell migration. **Methods:** We expressed Rab11 and Rab25 wildtype and mutant constructs in HeLa and MDA-MB231 cells and measured their effect on cell morphology, vesicle dynamics and migration behaviour. In prostate cancer samples we analyzed the expression of both GTPases. **Results:** Cells grown on fibronectin displayed a more stretched morphology when Rab11 was inactivated, whereas inactivation of Rab25 led to reduced stretching. Overexpression of both Rab11 and Rab25 accelerated cell migration. Analysis of vesicular movement revealed higher transport

efficiency in the inner cell compartment for Rab11 positive vesicles and in proximity to the membrane for Rab25 positive vesicles. Interestingly, we found Rab25 to be highly expressed in prostate cancer tissue. **Conclusion:** Taken together, our data suggest that Rab11 is mainly responsible for basal long-distance transport from the rear end to the front of the migrating cell, whereas Rab25 acts predominantly in the small-scale fast recycling within the tips of the cell. Our results further support the idea of Rab25 as a promoter of tumor development.

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Introduction

During cell migration, the constant flux of integrins and other surface receptors from the back of the cell to the leading edge is crucial for constant movement [1]. However, trafficking of cell surface receptors requires coordinated vesicular transport. Members of the Rab family of small GTPases have been shown to be major regu-

lators of intracellular membrane trafficking events [2-4]. Rab11 and Rab25 (also termed Rab11c) are closely related and have both been shown to play important roles in receptor recycling [5, 6]. The well-studied Rab11 regulates endosomal recycling through the trans-Golgi network and has been associated with the trafficking of a variety of different surface receptors, e.g. GLUT1, NPC1L1, transferrin receptor, TGF-beta and different integrins [7-13]. All Rab GTPases fulfil their specific function by cycling between a GTP bound active conformation and, after GTP hydrolysis, a GDP bound inactive state [14]. Indeed, Rab25 exhibits a special feature as it contains an activating mutation (Leucine instead of Glutamine at position 71) with a suggested persistence in the GTP bound state [15]. It can be presumed that this mutation yields a constitutively GTP-bound protein, although data regarding this are partially controversial [5]. The Rab25 mutation is reminiscent of the activating mutation in oncogenic Ras mutants [16]. Thus, Rab25 came into focus in terms of its involvement in tumorigenesis. In recent studies, two apparently opposing roles of Rab25 in tumor progression evolved. On the one hand, several groups proposed a tumor-promoting function of Rab25 and associated its overexpression with an increase in tumor cell aggressiveness and invasiveness. Rab25 was shown to interact with the beta1 subunit of alpha5 beta1 integrins and to increase the invasiveness of tumor cells by accelerating the integrin-recycling within the pseudopodial tips of the migrating cells [17]. High expression levels of Rab25 also dramatically increased the aggressiveness of ovarian and breast cancers both *in vitro* and *in vivo* [18]. Moreover, upregulated expression of Rab25 was detected in a gene expression analysis in prostate cancer cell lines and found to be associated with prostate oncogenesis [19]. Finally, Rab25 was found to be upregulated in gene expression signatures of invasive mammary carcinoma cells [20].

On the other hand, the labs of Rao and Goldenring showed a relation between loss of Rab25 expression and increased tumor development suggesting a role of Rab25 as a tumor suppressor: A loss of Rab25 expression was detected in some breast cancer tissue samples and in a newly established breast cancer cell line [21]. Re-expression of Rab25 led to an arrest of anchorage-independent growth and decreased *in vivo* tumor formation [21, 22]. Further, in *APC^{Min/+}* mice the loss of Rab25 increased the development of intestinal neoplasia and colorectal carcinoma [23]. These mutant mice carry a truncated *Apc* gene (adenomatous

polyposis coli) and have been described to develop multiple intestinal neoplasia (Min). These lesions only rarely develop into adenocarcinoma if no additional mutations occur. Thus, the results of this study suggest that Rab25 protects from tumor development. Interestingly, this effect went along with a decreased appearance of beta1 integrins on the lateral membranes in the villi. The authors suggested that loss of Rab25 may promote the transformation of epithelial cells by abrogating the trafficking of membrane molecules involved in the determination of cell polarity [23].

Actually, despite their well-studied functions described above, it is still unclear whether Rab11 and Rab25 exert redundant or complementary roles, how they interact and what is the relevance of the constitutively activating mutation in Rab25 in these processes. Here, we aimed to clarify similarities and differences in the impact of Rab25 and Rab11 on cell morphology, vesicle transport and migration of cancer cells with a focus on the influence of the activating mutation in Rab25. Moreover, we aimed to monitor the expression of Rab11 and Rab25 in clinical cancer tissue samples.

Materials and Methods

Generation of constructs and mutants

The coding DNA sequences for Rab11 and Rab25 wildtype were amplified from a human cDNA library derived from HepG2 cells (provided by P. Bayer, University of Duisburg-Essen). For PCR amplification, the following primers were used:

Rab11-EGFP-for
5' CGG AAT TCA ATG GGC ACC CGC GAC GA 3'
Rab11-EGFP-rev
5' CGG GAT CCT TAG ATG TTC TGA CAG CAC T 3'
Rab25-EGFP-for
5' CGG AAT TCG ATG GGG AAT GGA ACT GAG G 3'
Rab25-EGFP-rev
5' CGG GAT CCT CAG AGG CTG ATG CAA CAG 3'

The cDNA sequences were cloned into a pEGFP_C1 vector (Clontech) using restriction enzymes EcoRI and BamHI following standard protocols. After successful cloning, specific point mutations were inserted by QuikChange site-directed mutagenesis. For Rab11 we generated constitutively active Rab11 S20V (GTPase deficient) and the constitutively inactive Rab11 S25N (GTP-binding deficient) that had previously been described [6, 24].

When compared to switchable GTPases like Rab1A, Rab6a and Rab11a, the Rab25 wildtype protein contains a Q to L mutation in the highly conserved phosphate binding motif PM3 (WDTAGQ) of this protein family. The altered PM3

motif (WDTAGL) results in a GTP-hydrolysis-deficiency and is characteristic for constitutively activated GTPases, such as the commonly used constitutively activated mutant of Rab6 (Rab6 Q72L) [3]. Therefore, we generated a re-mutated “proto-wildtype” version, Rab25 L71Q. This mutant, according to sequence homologies, corresponds to an active GTPase with regular GTP-hydrolysis capability (see Fig. 1A). Based on this construct, we then generated a GTP-binding deficient mutant bearing the typical inactivating mutation, Rab25 L71Q/T26N. An overview of all constructs is given in Figure 1B. For mutagenesis, the following primers were used (mutated positions are bold):

RAB11 S20V for

5' GTT GTC CTT ATT GGA GAT **GTT** GGT GTT GGA AAG AGTA 3'

RAB11 S20V rev

5' TAC CTT TCC AAC ACC **AAC** ATC TCC AAT AAG GAC AAC 3'

RAB11 S25N for

5' CTG GTG TTG GAA AGA ATA ATC TCC TGT CTC G 3'

RAB11 S25N rev

5' CGA GAC AGG AGA TTA **TTC** TTT CCA ACA CCA G 3'

RAB25 T26N for

5' CAG GTG TGG GGA AGA ACA ATC TAC TCT CCC 3'

RAB25 T26N rev

5' GGG AGA GTA GAT TGT **TCT** TCC CCA CAC CTG 3'

RAB25 L71Q for

5' GAC ACA GCT GGC **CAG** GAG CGG TAC CGA 3'

RAB25 L71Q rev

5' TCG GTA CCG CTC CTG GCC AGC TGT GTC 3'

The mutagenesis reactions were performed with a Quikchange mutagenesis kit (Stratagene) according to the instructions of the manufacturer.

All constructs were sequenced to verify sequence correctness. Proper protein expression was verified in HeLa cells by fluorescence microscopy and Western Blot analysis (<http://www.uni-due.de/~hy0546/Kessler2012/>).

Transfection and measurement of cell stretching

HeLa Cells were grown on glass cover slips either uncoated or coated with fibronectin (1 mg / ml), poly-L-lysine (0.1 mg / ml) or collagen (1 mg / ml), respectively.

Transfections were done using Lipofectamine2000 (Invitrogen) according to the protocols of the manufacturer and with a DNA:Lipofectamine ratio of 1:3. Cells were grown for 24 hours after transfection with a medium exchange after 4-6 hours. Cells were then fixed and permeabilized using 3% PFA + 0.2% Triton-X100 and subsequently counterstained with Hoechst 33258 (Invitrogen) and Phalloidin-TRITC (Sigma) to stain the nucleus and the actin cytoskeleton, respectively.

Cells were imaged using a Zeiss Cell Observer fluorescence microscope equipped with the respective filter sets, an Apotome and an AxioCam MRm. Length and area of the cells were then measured using the Zeiss Axiovision 4.8 software. For each construct, a minimum of 60 cells were analyzed. Untransfected cells and EGFP empty vector transfected cells were evaluated as a reference.

Cell migration

MDA-MB231 breast cancer cells were transfected as described above. 22 hours after transfection, living cells were imaged for 18 hours with an interval of 10 minutes, both in fluorescence and phase contrast. From each movie, EGFP positive cells and, as a reference, untransfected cells were tracked manually using the Axiovision Tracking module (<http://www.uni-due.de/~hy0546/Kessler2012/>). Velocity, total distance, straight distance and tortuosity (=total distance/straight distance, a measure for the “tumbling” of the object) were measured. The percentual changes in straight distance and velocity that are depicted in Figure 1C were calculated from mean values of transfected cells relative to control cells.

Dynamics of vesicular structures

Hela cells were transfected as described above. After 22 hours, selected cells were imaged for two minutes each with maximum exposure speed. For further analysis, the Tracking module of the Axiovision Software package was used. In each cell that was analysed, up to 100 single vesicles were manually chosen in two different regions of the cell, an inner cell region (ICR) and an outer cell region (OCR; along the cell membrane and in the very tips of the cell). The tracking of the selected vesicles was performed by the software and for each vesicle velocity, total distance, straight distance and tortuosity were calculated. Up to 1600 different vesicles for each construct and cellular region were tracked and analysed. Examples for the definition of ICR and OCR including some exemplary tracks in each region are shown in Figure 2A.

Immunohistochemistry

Paraffin embedded tissue samples of acinar adenocarcinoma of the prostate from nine random patients were retrospectively retrieved from the archive of the Institute of Pathology and Neuropathology. Gleason scores of the nine samples were: 2+3, 3+2, 3+3 (4x), 3+4, 4+3 and 4+5. Representative haematoxylin- and eosin-stained sections were reviewed by a pathologist to confirm the diagnosis and its Gleason score in the tissue specimen used for further analysis. The samples were pre-treated by incubation in Zytomed Zuc028 citrate buffer (pH 6.0) at 98°C for 20 minutes. Staining of the slides was performed in the Zytomed Polymer System POLHRP-100. The antibodies against Rab25 (Abcam ab32004; dilution 1:800) and Rab11 (BD Bioscience 610657; dilution 1:200) were diluted in Zytomed Zuc025. The slides were counterstained with Haematoxylin (dilution 1:8) and embedded in Eukitt. Analysis of the slides was done using a Leica DMIRB microscope equipped with a Zeiss AxioCam HRC color camera and Axiovision 4.7 software.

Statistical data analysis

Data analysis was done using the statistical software R (<http://www.r-project.org/>). We performed Wilcoxon signed-rank-tests to compare the different datasets with each other, due to the fact that almost all datasets are not normally distributed with regard to Shapiro-Wilk tests. We calculated standard deviation (SD) values.

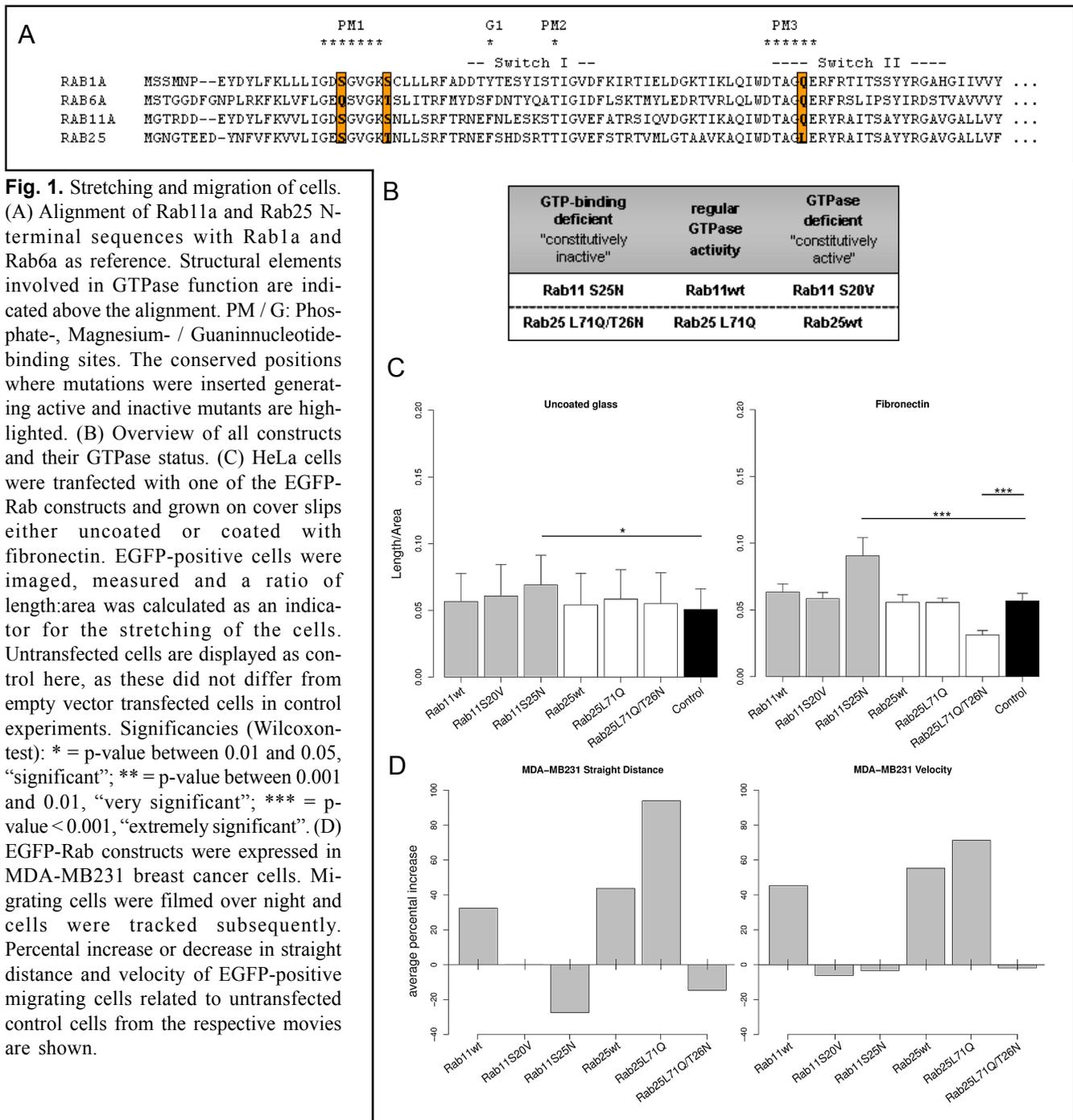


Fig. 1. Stretching and migration of cells. (A) Alignment of Rab11a and Rab25 N-terminal sequences with Rab1a and Rab25 and Rab6a as reference. Structural elements involved in GTPase function are indicated above the alignment. PM / G: Phosphate-, Magnesium- / Guaninnucleotide-binding sites. The conserved positions where mutations were inserted generating active and inactive mutants are highlighted. (B) Overview of all constructs and their GTPase status. (C) HeLa cells were transfected with one of the EGFP-Rab constructs and grown on cover slips either uncoated or coated with fibronectin. EGFP-positive cells were imaged, measured and a ratio of length:area was calculated as an indicator for the stretching of the cells. Untransfected cells are displayed as control here, as these did not differ from empty vector transfected cells in control experiments. Significancies (Wilcoxon-test): * = p-value between 0.01 and 0.05, "significant"; ** = p-value between 0.001 and 0.01, "very significant"; *** = p-value < 0.001, "extremely significant". (D) EGFP-Rab constructs were expressed in MDA-MB231 breast cancer cells. Migrating cells were filmed over night and cells were tracked subsequently. Percentual increase or decrease in straight distance and velocity of EGFP-positive migrating cells related to untransfected control cells from the respective movies are shown.

Results

Effects of Rab11 and Rab25 overexpression on cell morphology

To gain insight into the effects of Rab11 and Rab25 on cell morphology, wt and mutant constructs of both GTPases were expressed in HeLa cells to study suggested differential effects. HeLa cells were

chosen because they exhibit a large and widespread morphology and can easily be measured. Length and area of transfected HeLa cells were determined and a ratio of length:area was calculated as a measure for cell stretching: An increased ratio was indicative for an increased cell stretching.

When cells were grown on uncoated glass cover slips, only overexpression of inactive Rab11

S25N led to a slight increase in cell stretching. In contrast, when grown on fibronectin, cells were significantly more stretched when inactive Rab11 S25N was overexpressed whereas overexpression of inactive Rab25 L71Q/T26N significantly decreased cell stretching (Fig. 1C). In control experiments, untransfected cells did not differ significantly from empty vector transfected cells (data not shown), thus in the Figure only untransfected cells are presented as control. The experiments were also performed on collagen and poly-L-lysine matrices, but no significant differences compared to uncoated glass cover slips were found (data not shown).

Cell migration

The impact of Rab11, Rab25 and their mutants on cell migration was studied in MDA-MB231 breast cancer cells. These cells exhibit a well traceable migration behaviour and turned out to be strong enough to resist the overall stressful experimental procedures. When MDA-MB231 cells were transfected with Rab11, Rab25 or their mutants, respectively, the overexpression of both Rab11 and Rab25 wildtype constructs led to an increase in velocity and distance of cell migration in comparison to untransfected control cells (Fig. 1D). The proto-wildtypic GTPase-active mutant Rab25 L71Q enhanced migration speed and distance at even a higher level compared to Rab25wt. The expression of the inactive mutants Rab11 S25N and Rab25 L71Q/T26N slightly decreased the straight distance and to a lesser extent the velocity of migration, resulting in a less directed migration. The expression of the constitutively active Rab11 S20V had nearly no effect on distance and speed (Fig. 1D).

Dynamics of vesicular structures

EGFP-positive vesicular structures were imaged and tracked in HeLa cells transfected with Rab11 and Rab25 wt and mutant constructs. HeLa cells showed good expression levels for all constructs as well as clearly distinguishable patterns of vesicle distribution and were thus chosen for these experiments. Vesicles were traced in two separate regions of the cell: An inner cell region (ICR) and an outer cell region (OCR) along the membrane and within the very tips of the cell (Fig. 2A).

Comparing the wildtypes, Rab11-positive structures covered a longer straight distance in the ICR than Rab25-positive structures (Fig. 2B). In contrast, in the OCR, Rab25-positive structures covered a little

longer distance than Rab11-positive vesicles. The difference between straight distances covered within the ICR and the OCR was greater for Rab11 wt than for Rab25 wt (Fig. 2B).

Interestingly, when Rab25 was mutated to the proto-wildtypic GTPase-active form L71Q, the straight distance covered within the ICR significantly increased. Vice versa, the constitutive activation of Rab11 (S20V) led to a significant increase in the covered distance within the OCR. Compared to the respective wildtype GTPases, the difference between transport in the ICR and the OCR was decreased for Rab11 S20V and increased for Rab25 L71Q. Additionally, both constitutively active GTPases (Rab11 S20V and Rab25 wt) showed significantly reduced tumbling within the outer cell regions, whereas the GTPase-active forms (Rab11 wt and Rab25 L71Q) showed only slight differences in tumbling between the inner and outer cell region (Fig. 2B).

Expression of Rab11 and Rab25 in prostate cancer tissue samples

We also performed an immunohistochemical analysis of Rab11 and Rab25 expression in nine tissue specimens of prostate cancer patients with different Gleason scores. We compared cancerous areas, normal glands and connective tissue within the same samples and checked for the presence or absence of the proteins in the different tissue areas. As shown in Figure 3 for a representative patient sample (Gleason 4+3), Rab11 signals were detected in all cell types of the tumor tissue specimens, although at a relatively low level. In contrast, Rab25 staining yielded strong signals in all epithelial cells of normal and cancerous prostatic glands but was hardly detectable within the smooth muscle cells or the connective tissue. No significant differences in this distribution between samples with different Gleason scores were visible.

Discussion

Cell stretching

Our observations might be seen as a hint for a predominant function of Rab25 in the extension of the cell front and of Rab11 in the retraction of the back of the migrating cell: When Rab11 is inactive, the cell cannot retract its end with the same velocity as it extends its front. Thus, the cell becomes stretched. Vice versa, when Rab25 is inactive, the

Fig. 2. Dynamics of vesicular structures. HeLa cells transfected with different Rab constructs were imaged for 2 minutes. Subsequently, EGFP-positive vesicular structures were tracked. (A) Example for the definition of vesicles within the inner cell region (ICR) and the outer cell region (OCR). Details show exemplary tracks within each region. (B) Straight distance and tortuosity of vesicular structures positive for Rab11wt, Rab11S20V, Rab25wt or Rab25L71Q within the ICR and the OCR. Selected significancies between ICR and OCR as well as between wildtype and respective mutant are depicted. Significancies (Wilcoxon-test): * = p-value between 0.01 and 0.05, “significant”; ** = p-value between 0.001 and 0.01, “very significant”; *** = p-value < 0.001, “extremely significant”; all comparisons that are not further specified are not significant.

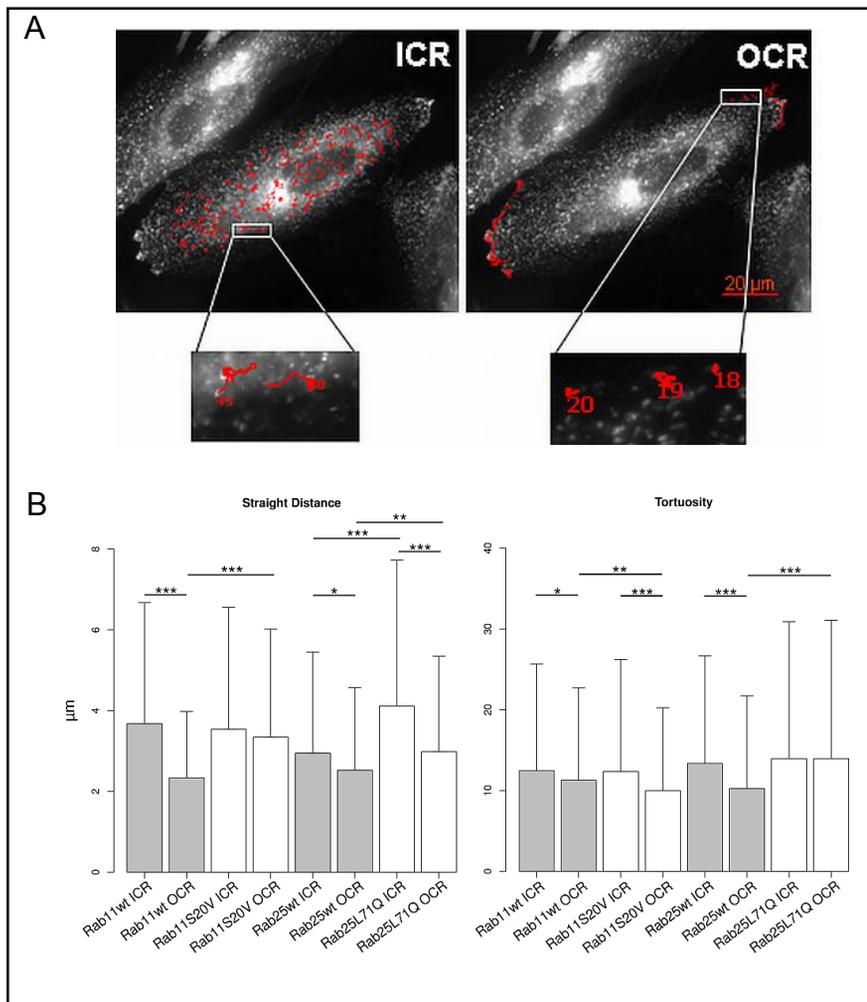
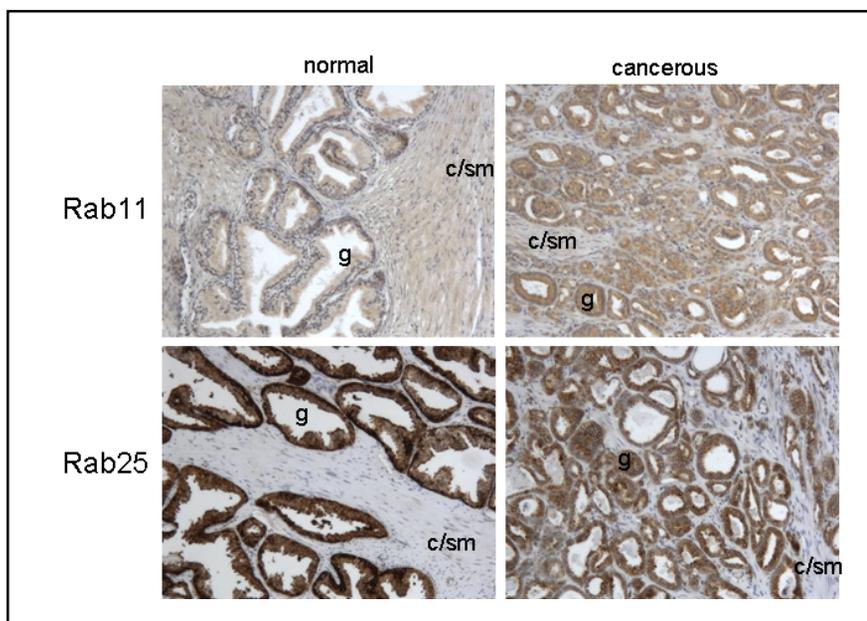


Fig. 3. Immunohistochemistry of prostate cancer samples. Immunohistochemical reaction of Rab11 and Rab25 antibodies in normal and cancerous prostatic glands and stromal cells of a prostate cancer tissue sample. Cancerous and normal areas within the same section were compared. The Gleason score of the sample shown here is 4+3. Note the distinct cytoplasmatic staining while nuclei are generally spared. All images taken at 100x magnification. g = gland; c/sm = connective and smooth muscle tissue.



velocity of extension of the front is lower than the retraction of the rear and, as a result, the cell is

rounded up. This effect is obviously dependent on the interaction with a fibronectin matrix. The observation

that differences in cell stretching are only visible in cells grown on fibronectin suggests that they probably depend on the transport of fibronectin-binding (beta1) integrins. This would partially corroborate earlier studies that showed a direct interaction between Rab25 and beta1 integrin, but could not detect any binding of beta1 integrin to Rab11 [17].

Cell Migration

Both Rab11wt and Rab25 wt exhibited a promoting effect on tumor cell migration and the re-mutated Rab25 L71Q with suggested restored GTPase-activity had an even more pronounced effect than the respective wildtype. A reason for this might be the limited dynamics of the constitutively GTP loaded Rab25 wt that restricts a continuous switching between an active and an inactive conformation. The same would be true for Rab11 and its constitutively active mutant Rab11 S20V: the latter exhibits only a negligible effect on cell migration. The fact that both GTPase-deficient mutants (Rab11 S25N and Rab25 L71Q/T26N) cause a decrease of the straight distance covered by the migrating cell, but do not affect migration velocity, reveals that not the speed but the directionality of migration is affected by the inactivation of either of the small GTPases.

The results of our cell migration experiments differ from an earlier publication, where no changes in speed, persistence or directionality were detected when migration of A2780 human ovarian carcinoma cells overexpressing Rab25 was analysed on 2D plastic surfaces [17]. A possible explanation for this inconsistency might lie in the use of cell lines derived from different types of carcinoma, i.e. breast and ovarian, and thus with a distinct genetic background.

Vesicle dynamics

Using an innovative experimental approach we were able to monitor the dynamics of vesicular structures in different regions of the cell and to address questions that were raised by the work of Caswell and others [17]. We collected interesting results that hint to a certain functional partitioning of Rab11 and Rab25 and to spatial differences in the impact of both proteins on vesicle dynamics. Rab11 seems to regulate transport processes more efficiently within the ICR than Rab25 does. Due to reduced vesicle tumbling, the constitutively active Rab25 might more effectively promote vesicle trafficking close to the membrane. This functional separation seems to be rooted in the

activating mutation that leads to a reduced tortuosity of vesicular traffic close to the membrane and a decreased difference between ICR and OCR transport, as could be shown by the introduction of the respective mutations. An explanation for this might be the same as already mentioned above: The GTP-locked GTPases are unable to dynamically cycle between active and inactive forms, which may not be required for trafficking over smaller distances, but may be necessary for continuous transport processes over longer distances.

Generally, the results of these experiments are consistent with the results of Caswell et al. [17] who showed that Rab25 is responsible for small-scale recycling of integrins within the exceeding lamellipodia of the cell.

Immunohistochemistry

Generally, our results corroborate the results of Goldenring et al. [15] who reported an epithelial expression of Rab25 and a ubiquitous expression of Rab11. With respect to prostate cancer, we show Rab25 to be highly expressed in the epithelial cells that are related to tumor initiation and progression. Hence, this would support a role of Rab25 as a promoter of tumor development in prostate cancer. In line with this assumption an expression profiling published by Calvo et al. [19] revealed that Rab25 is upregulated in prostate cancer cell lines and was suspected to play a role in tumor progression. However, here we noticed no significant differences between Rab25 immunoreaction in cancerous and normal glands, which may be due to the fact that we only analyzed benign glands within cancer tissue samples. A comparison with glands from non-cancerous prostate tissue would allow to verify putative differences in the future.

Concerted action of Rab11 and Rab25

Taken together, in the experiments described in the present manuscript, we could demonstrate that Rab11 and Rab25 both increase the velocity and distance of cell migration, have differential effects on cell morphology, exhibit a certain partitioning in vesicle dynamics, and show different expression patterns in prostate tumor tissue. Altogether, these results point to a spatially and functionally diverted action of Rab11 and Rab25. Rab11 presumably acts predominantly in long distance transport of vesicles and might influence the retraction of the rear end of the cell, whereas Rab25 seems to have its major function in rapid

small-scale recycling within the very tips at the extending front. The action of Rab11 seems to fulfil the more basic requirements whereas Rab25 action can expand this basal function, e.g. when rapid recycling during the extension of the cell front is needed. As a consequence, increased expression of Rab25 found in tumor tissues may increase the migratory potential of the malignant cells.

The results explained here are depicted in a diagram highlighting our hypothesis of a concerted action of Rab11 and Rab25 (<http://www.uni-due.de/~hy0546/Kessler2012/>).

Rab25 and its role in tumor progression: does regulation by RCP cause different outcomes?

At first sight, our model supports the view of Rab25 as a tumor promoter, driving migration and invasion by adding rapid recycling within invasive structures and thus enhancing the basal action of Rab11. Nevertheless, strong arguments in the literature also support the idea of Rab25 acting as a tumor suppressor. It might be possible, that the influence of Rab25 on cell function may largely depend on the genetic background, the tissue origin, or both. Indeed, an overexpression of Rab25 has been reported for ovarian cancers whereas a loss of Rab25 was monitored in colorectal carcinoma [18, 23]. However, for breast cancer cells, Rab25 overexpression has been linked either to tumor promotion or to tumor suppression [18, 21]. Thus, the effect of Rab25 on tumor initiation and progression may not only depend on the GTPase itself, but also on its interplay with accessory proteins or its mode of regulation in the respective tumor cell.

In this regard, it has been suggested recently, that the role of Rab25 in tumor progression may be linked to the status of the Rab-coupling protein RCP [25]. RCP belongs to a class of proteins termed Rab11-family interacting proteins (Rab11-FIPs). It binds to Rab11a, Rab11b and Rab25 [26, 27] and has been shown to regulate endocytic protein sorting and integrin recycling driving cell migration [28, 29]. In association with Rab11 it plays a role in axonal trafficking of beta1 integrins [13]. It is amplified in breast cancer and is supposed to promote tumorigenesis, most probably in concert with Rab25 [30, 31]. Consequently, a tumor promoting effect of increased Rab25 levels may only occur in combination with high levels of RCP [25]. Indeed, mRNA levels of

Rab25 and RCP have been shown to correlate in breast cancer [31]. Low levels of RCP may lead to a competition in binding of RCP to Rab25 or other GTPases like mutant Ras, which in turn might minimize their oncogenic effects [25]. The scenario becomes even more complex as five different isoforms of RCP have been described to date [32]. The expression levels of the five RCP isoforms in different cells and tissues and their role in the regulation of Rab25 in tumor progression remain to be defined.

Other Rab GTPases in cell movement: Towards the whole picture

Although our model is based on a variety of data gathered by us and others, it has still to be considered a very simplified model that may only serve to explain the (inter)action of Rab11 and Rab25. The whole picture of recycling processes of integrins and other surface receptors is far more complex. Several Rab GTPases besides Rab11 and Rab25 have been shown to be involved in the different stages of recycling and endosomal trafficking. Rab5 is one of the best studied Rab proteins in endosomal trafficking. It is involved transport from the plasma membrane to early endosomes and in homotypic early endosomal fusion [33, 34]. Along with Rab4 and Rab11 it has been shown to form distinct microdomains on the vesicle surface [35]. These microdomains consist either of Rab5 alone, Rab4 and Rab5 or Rab4 and Rab11 and thereby provide the base for functional diversity of different vesicle populations [35]. In contrast, late endosomes display microdomains consisting of Rab7 and Rab9 [36].

Focussing on integrin recycling, some more Rab proteins come into play. Rab21 is a regulator of alpha5beta1 and alpha2beta1 integrin trafficking and thereby plays a role in adhesion-dependent processes like migration and cytokinesis [37, 38]. Recently Rab1a, that has extensively been studied in terms of ER-to-Golgi- and intra-Golgi-trafficking, has also been shown to influence cell migration by controlling the recycling of beta1 integrins [39].

Thus, not only regulatory mechanisms for Rab25 itself remain to be solved, but also its interplay with other Rab GTPases in the complex network of vesicular trafficking processes has to be a major aim in future studies to complete the whole picture.

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