Regulation of cell migration by dynamic microtubules

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Abstract

Microtubules define the architecture and internal organisation of cells by positioning organelles and activities, as well as by supporting cell shape and mechanics. One of the major functions of microtubules is the control of polarized cell motility. In order to support the asymmetry of polarized cells, microtubules have to be organised asymmetrically themselves. Asymmetry in microtubule distribution and stability is regulated by multiple molecular factors, most of which are microtubule-associated proteins that locally control microtubule nucleation and dynamics. At the same time, the dynamic state of microtubules is key to the regulatory mechanisms by which microtubules regulate cell polarity, modulate cell adhesion and control force-production by the actin cytoskeleton. Here, we propose that even small alterations in microtubule dynamics can influence cell migration via several different microtubule-dependent pathways. We discuss regulatory factors, potential feedback mechanisms due to functional microtubule-actin crosstalk and implications for cancer cell motility.

Keywords

cell migration; cell motility; microtubule dynamics; microtubule associated proteins; focal adhesion turnover; actin cytoskeleton

1. What is microtubule dynamics?

The microtubule cytoskeleton serves various vital cellular functions. Microtubules provide the tracks for intracellular long-distance transport, positioning of organelles and intracellular activities, thereby defining interphase cellular architecture and ensuring precise chromosome segregation in mitosis. Microtubules also function to support cell shape and mechanics due to their ability to resist high compressive loads [1]. Microtubules are composed of alpha and beta tubulin heterodimers that bind head-to-tail to form protofilaments, 13 of which form a hollow

tubule. This architecture gives microtubules an intrinsic polarity with assembly and disassembly occurring exclusively at their ends. The observation that in a population of microtubules, some ends grow while others shrink led to the GTP cap model of dynamic instability [2]. In this model, GTP tubulin incorporates at the end of the microtubule and forms a stabilising GTP cap at the growing end. Subsequent GTP hydrolysis leads to the lattice of the microtubule consisting mainly of GDP tubulin, which prefers an outward bent conformation and thus promotes microtubule depolymerisation when exposed at the microtubule end [3]. Thus each microtubule end switches between periods of growth and shrinkage, which are governed by the presence or loss of the GTP cap [4]. The two ends of a microtubule are not equal. The plus end, where beta tubulin is exposed, grows and shrinks faster and is thus also called the dynamic end.

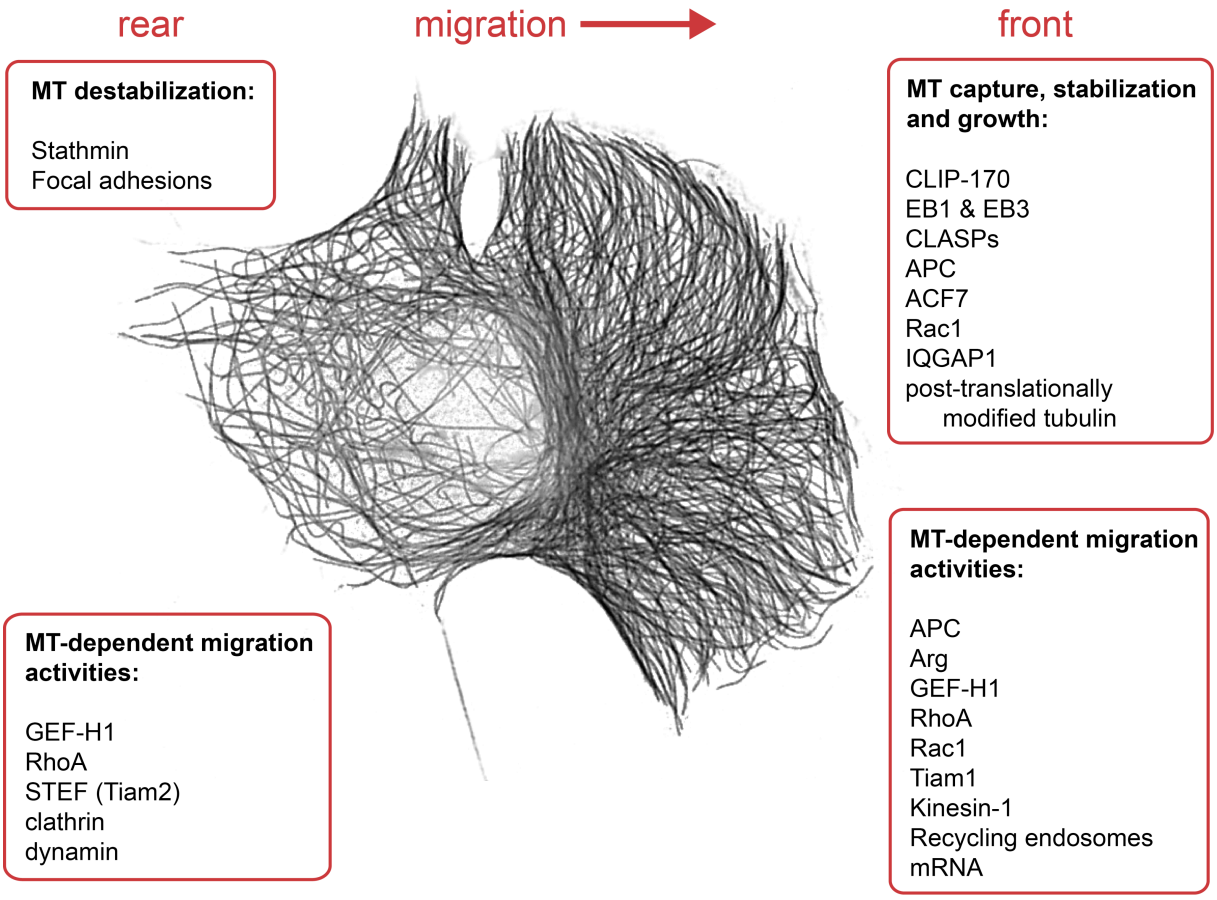
2. How is microtubule dynamics regulated?

In cells, the microtubule minus end is usually embedded in the main microtubule-organizing centre (MTOC) and does not grow [5], while the plus end explores the cellular space and tends ultimately to come into contact with the cell edges. Microtubules do not show stochastic switching between growth and shrinkage in cells. Instead, transitions appear to be spatially and temporally regulated. Microtubules generally grow persistently in the cytoplasm and most catastrophes are induced at the cell edges [6, 7]. This results in most microtubules reaching the cell edge, thus ensuring efficient cargo transport. At the cell edges, microtubule ends often dwell for some time. This state usually involves fast transitions between growth and shrinkage phases, even though microtubules doing this are frequently referred to as paused or captured [8]. The observed dynamics of microtubule plus ends in cells suggests extensive regulation. Many microtubule regulatory factors are known, some of which promote assembly and some of which induce disassembly (see [9] for a recent review). Mechanistically, microtubule dynamics can be regulated in various ways, for example XMAP215 family proteins are thought to catalyse microtubule growth by stabilising a transitional state and thus favour tubulin subunit incorporation [10], while Kinesin-13s probably induce microtubule disassembly by promoting a bent conformation of tubulin subunits, thereby destabilising lateral protofilament interactions [11, 12]. The fast transitions between growth and shrinkage observed at the cell boundaries are likely to require the action of both a catastrophe inducer and a rescue factor, whose activities are somehow coordinated. How such regulatory crosstalks operate remains to be understood and is a major challenge for the future. Recent perturbation experiments suggest that CLASPs can act as cortical rescue factors [13] and that EB3 is involved in the regulation of both catastrophe induction as well as rescue promotion at cortical sites [14].

3. Microtubule dynamics is asymmetric to polarize a motile cell

In order to support asymmetry of cellular activities, the microtubule network itself has to be asymmetric. Microtubule asymmetry in a motile cell includes both asymmetric microtubule distribution and microtubule dynamics. In most cells, like fibroblasts and neurons, more microtubules extend to the cell front than to the cell rear. Such difference is due to a combination of cell front-specific activities that result in increasing microtubule numbers and selective de-stabilization of microtubules at the cell rear (see Figure 1). Front activities include selective capture and stabilization of microtubules at cortical sites [13-17], selective support of persistent microtubule growth by local Rac1-dependent tubulin polymerization [18], inactivation of catastrophe factors like stathmin [19], and the asymmetric nucleation of microtubules at non-centrosomal Golgi-associated MTOCs [20]. Moreover, in many cases microtubule motors contribute to the polarized organization of the microtubule network either by transporting or by crosslinking microtubules. Recent studies implicate a collaborative effort of kinesin-5 and

Figure 1. Polarized regulation of microtubule dynamics and molecular factors asymmetrically regulated and/or positioned by microtubules at the front or rear of motile cell. Background: asymmetric microtubule cytoskeleton of a Swiss 3T3 fibroblast visualised by tubulin immunostaining.



kinesin-12 in neuronal outgrowth and growth cone guidance, potentially by using their microtubule cross-bridging activity to prevent microtubules invading the growth cone equally, thus supporting asymmetry [21-23]. In contrast, kinesin-1 can actively slide microtubules along each other to support the formation of parallel microtubule arrangements in cellular protrusions in multiple cell types [24].

Long-lived microtubules that extend to the cell front are often posttranslationally modified [25]. It is thought that stable microtubules accumulate tubulin modifications such as acetylation and detyrosination over time [26], but also that tubulin acetylation protects microtubules from depolymerisation and thus may reinforce stability [27, 28]. Tubulin modifications selectively increase the affinity of certain molecular motors to their tracks (e.g. kinesin-1 [29, 30]) and could thereby serve as signposts to facilitate directional transport to the cell front. At the rear part of the cell, frequent catastrophes [31] are observed. Preferential microtubule destabilization at the cell rear is likely to be triggered by an excess of active stathmin that is inhibited by phosphorylation at the cell front but not in the rear [19]. Catastrophes are also specifically induced at adhesion sites [32], many of which are positioned in the trailing part of a cell. On the other hand, early adhesions at the cell front and their surroundings have been implicated in capturing microtubules and increasing their life times [33, 34], and how these two opposite activities are differentiated is not yet known. One possible hypothesis predicts the existence of a regulatory mechanism that results in catastrophe-inducing adhesions, which are possibly mature adhesion sites that are associated with paxillin [32], and stabilizing adhesion sites of a different, to-be-defined composition. In this case, these two groups of adhesions should be differentially distributed in a polarized motile cell.

Even though more microtubules grow towards the cell front, the density of microtubules close to the cell cortex is lower at the protruding front than at the retracting rear [35]. This is probably caused by the speed of membrane protrusion exceeding that of microtubule polymerisation together with the active rearward transport of microtubules by the actin retrograde flow [36]. Thus, differences in microtubule dynamics between the cell front and the cell rear are quite significant on their own and, in addition, underlie the differences in microtubule distribution observed.

4. Microtubules regulate cell motility

The role of microtubules in cell migration has been established since Vasiliev and Gelfand observed that fibroblasts in culture cease motility when treated with the microtubule depolymerising drug colcemide [37]. Subsequent studies showed that not only full microtubule

depolymerisation but also abolishing microtubule dynamics using Taxol or low doses of nocodazole interferes with cell motility [38]. The major question arising from these studies was which mechanistic component of cell migration is regulated by dynamic microtubules. Directional cell migration requires protrusion of the cell front and retraction of the rear, processes that are driven by actin polymerisation and acto-myosin contraction. Furthermore, traction is provided through integrin-mediated links between the extracellular substrate and the cytoskeleton. These activities do not require microtubules per se (see also section 7). However, many processes essential to cell motility are regulated by microtubules and depend on distinct modes of microtubule dynamics (see Figure 1).

One group of such processes relates to the assembly of the actin cytoskeleton. Microtubules affect actin-driven leading edge protrusion by multiple pathways. Microtubule polymerization can activate the small Rho GTPase Rac1 [39], which is thought to occur through the guanidine exchange factor (GEF) activity of TIAM1 [40] or STEF (TIAM2) [41]. Several microtubule plus end binding proteins (+TIPs) including CLIP170 [15], APC [16] and CLASPs [42] interact with IQGAP1, an effector of Rac1 and Cdc42, that is thought to coordinate Arp2/3 and formin-dependent actin nucleation activities at cell protrusions (for a review see [43]). Assembly of actin filaments into larger structures and their functioning both at the cell front and cell rear depends on myosin II contractility regulated by RhoA signalling. RhoA, in turn, is locally controlled by GEF-H1 (Lfc) [44]. This molecule is the best-described player of microtubule-dependent actin regulation, as it is inactive when scaffolded to the microtubule lattice and is specifically activated by microtubule depolymerisation [45, 46].

The second group of microtubule-dependent processes that modulate cell migration relates to focal adhesion turnover. Microtubules directionally grow toward and target focal adhesions, thereby promoting their disassembly [33, 47]. Guidance of microtubule growth towards focal adhesions is thought to be mediated by the spektraplakin ACF7, which crosslinks microtubules and actin [48, 49]. Microtubule-induced focal adhesion disassembly is, at least partially, based on microtubule-stimulated dynamin and clathrin-dependent endocytosis of adhesion components [50, 51]. One the other hand, microtubule-dependent regulation of focal adhesions depends on activators of small GTPase-dependent pathways, such as STEF (TIAM2) [41]. Whilst the exact mechanism whereby microtubules trigger this pathway is not known, several sets of data indicate that local regulation of microtubule dynamics is important for focal adhesion disassembly. For example, focal adhesion disassembly occurs upon repetitive microtubule targeting [35] that involves multiple local microtubule catastrophes at the adhesion site and subsequent rescues in adhesion proximity [32]. Moreover, adhesion turnover is diminished upon

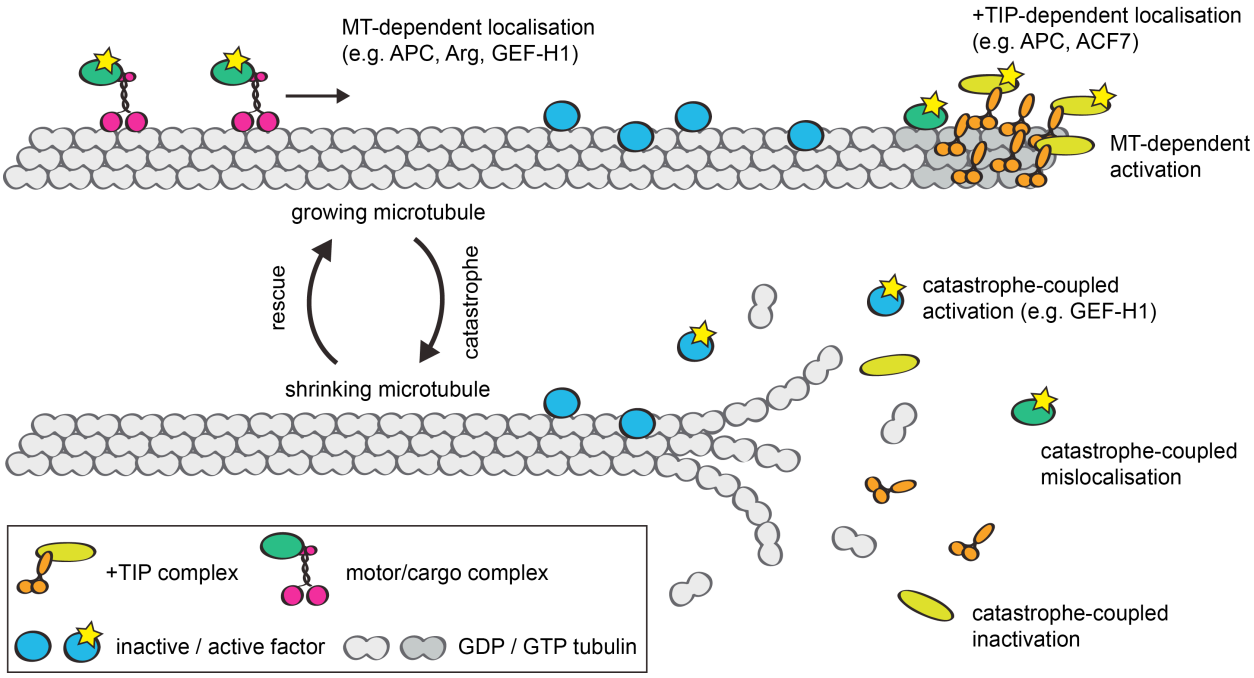
inhibition of deacetylase HDAC6 that leads to increased tubulin acetylation and suppression of microtubule dynamics [28].

Finally, efficient cell migration requires microtubule-dependent delivery of post-Golgi carriers [52, 53], recycling endosomes [54], mRNA [55] and other functional entities to the protruding cell edge. Microtubule-driven intracellular transport supports both actin cytoskeleton organization (e.g. the localisation of mRNA encoding Arp2/3 subunits [55]) and focal adhesion turnover through kinesin-1 activity [56]. Regulation of adhesion turnover by trafficking may occur via integrin recycling as part of recycling endosome trafficking (see [57] for review) since kinesin-1 has been implicated as an essential motor for recycling endosomes [58]. Furthermore, in macrophages microtubules regulate the turnover of invasive ventral actin protrusions, called podosomes. This regulation involves activity of the kinesin-3 family member KIF1C [59] and the kinesin-9 family member KIF9 [60] although the cargos that are delivered remain to be identified. Taken together, these evidences indicate that activity of microtubule-dependent molecular motors and availability of suitable microtubule tracks for motor movement is an essential requirement for directed cell migration.

5. The overall state of microtubule dynamics regulates multiple molecular factors

The set of factors that transduce microtubule signals to the cell migration machinery can be roughly divided into three subsets: those delivered by microtubule motors, those temporarily concentrated as components of the +TIP complex and those accumulated along the whole microtubule lattice. The activity of members of each group strongly depends on microtubule dynamics (see Figure 2). Microtubule motors, such as kinesin-1, prefer reliable tracks composed of post-translationally modified tubulin [29] or associated with specific stabilizing MAPs (e.g. ensconsin [61]) while other MAPs can reduce motor attachment [62]. +TIPs (see [63] for a recent review) can concentrate their activities locally at consistently growing microtubule ends, or be released acutely for action at a site of induced microtubule catastrophe. Certain factors of potentially high significance, like APC, possibly depend on both kinesin motor activity [64] and interaction with the +TIP complex [65] for accumulation at the microtubule plus end. Microtubule lattice binding proteins such as GEF-H1, can be sequestered and inactivated by a growing microtubule or, similarly to +TIPs, be acutely released and activated by a catastrophe [45, 46]. Other microtubule lattice binding active proteins, such as tyrosine kinase Arg [66], may be localised by growing or stable microtubules but diffuse away upon catastrophe. Thus, overall changes in microtubule dynamics, such as an increase in microtubule dynamicity or, alternatively, microtubule stabilization, could cause a distinct signalling response by

Figure 2: Putative mechanisms of microtubule dynamics-mediated control of protein localisation and activity.



changing the status of all the above-mentioned factors. Additionally, an overall shift in microtubule dynamics properties may alter the distribution of microtubule dynamics events in the cell, so as to make it more or less asymmetric. Altogether, these ideas prompt the hypothesis that mis-regulation of microtubule dynamics by generic means would produce a system-level response that alters cell polarity and migration via multiple pathways.

6. Microtubule-dependent alteration of cell migration implies changes in microtubule dynamics

Various microtubule-associated proteins (MAPs) have been implicated in the regulation of cell migration direction or the polarisation of cells. In melanoma cells, loss of EB1 reduces lamellipodial protrusion, velocity and persistence of cell migration [67]. APC also promotes cell front protrusion [68]. That the localisation of APC to microtubule ends depends on EB1 [65, 69] suggests that APC acts downstream of EB1. CLASPs are required for directionally persistent cell migration [53, 70] as well as to contribute to the formation of stable microtubules that accumulate acetylated and detyrosinated tubulin [71]. ACF7 has been shown to contribute to the localisation of CLASP2 [70], but also to be involved in epidermal migration directly using its actin-regulated ATPase function, which is required for targeting microtubules to focal adhesions [48]. Depletion of the microtubule-associated tumor suppressor RASSF1A results in extreme

protrusion activity and excessive migration in HeLa cells [72]. In neurons, MAP1b regulates axonal outgrowth through GEF TIAM1 [40]. Phosphorylation of CLIP-170 by the energy-sensing kinase AMPK regulates the rapid turnover of CLIP-170 at microtubule ends, which is important for fast microtubule growth, microtubule stabilisation, focal adhesion turnover and directional cell migration [73].

It is likely that all these functions of microtubule-associated proteins relate to their function in regulating microtubule dynamics. All MAPs implicated in cell migration thus far stabilise microtubules: EB1 and APC have been shown to act downstream of Rho and mDia in the stabilisation of microtubules to the front of migrating cells [74], EB1 and EB3 promote persistent microtubule growth in the cytoplasm [75], and APC stabilises microtubules by promoting growth, slowing shrinkage and decreasing transition frequencies [76]. MAP1b stabilizes microtubules and serves as a major scaffolding factor for microtubule-related activities in neurons [77, 78]. RASSF1a acts as a microtubule lattice-associated MAP acting in conjunction with small GTPase RAN to cause microtubule over-stabilization [79]. CLIP-170 and CLASPs possess rescue factor activity [80-82] that increases the cortical dwell time of microtubules [13].

A loss of stable (cold or drug-resistant) microtubules is frequently observed when these microtubule stabilisers are deleted from cells [70, 74, 77]. Although each of these proteins enhances microtubule stability by a specific mechanism, it is plausible to suggest that disturbance of this regulation would cause similar changes in overall microtubule dynamics. As we discussed above, such changes could result in a system-level disturbance in cell polarity and motility via multiple microtubule-dependent pathways.

7. Cell-type specificity or: size does matter

That said, paradoxically, none of the major motility processes of the cell absolutely require microtubules. Actin polymerization, focal adhesion turnover and delivery of post-Golgi carriers to the cell edge can proceed in the absence of microtubules or their proper dynamics. Rather, the polarisation of motility, in other words, the asymmetric distribution of motility processes requires microtubule regulation. Thus the main function of microtubules is to manage the overall organization and proper positioning of multiple activities within a cell in order to enable the persistent directional relocation of the whole cell. Microtubule control is more important for large cells than for small ones. A large number of studies have sought to understand how relevant microtubule control is for motility of diverse cell types. An often-cited example of a cell which can move directionally without microtubules is a fish keratocyte [83]. Similarly, directional migration of small chemotactic cells of hematopoetic origin is not abolished in the absence of

microtubules. For example, neutrophils can efficiently polarize and initiate movement in the absence of microtubules [84] though the efficiency and directional persistence of migration toward the chemo-attractant is decreased [85]. Similarly, T cells reduce but do not entirely lose the directionality of their migration if microtubules are destroyed [86]. Thus it appears that small cells can overcome a lack of microtubule-based cellular organization better than large ones. In particular, directional trafficking of required components can be more easily compensated in smaller cells where actin-based transport or diffusion can provide a sufficient supply. In simple systems, a persistent leading edge can be maintained after initial symmetry breakage by actin polymerization and acto-myosin contractility without additional stimulation [87, 88]. It is likely that such mechanoregulatory response underlies migration of small cells without microtubules [85-87, 89]. Moreover, small cells do not employ complicated adhesion machinery. Migration of immune cells is integrin-dependent but these cells likely don't form distinct adhesion sites (see [90-92] for reviews). Fish keratocytes have transient, dot-like adhesions [93], that are probably capable to disassemble without additional microtubule-introduced stimuli just like nascent focal complexes in bigger cells. In contrast, large systems like fibroblasts, motile epithelial cells and neurons are rendered disorganized and unable to move directionally in the absence of proper microtubule-mediated management of adhesion turnover, actin dynamics and membrane trafficking.

8. Crosstalk between actin and microtubule cytoskeleton

To describe the influence of microtubule dynamics on cell motility, we present a simple model whereby MAPs regulate microtubule dynamics, which, in turn, influences actin assembly, adhesion and cell polarity. This model is incomplete because does not take into account various possible feedback and cooperativity effects. The polarity of the microtubule network is to a large extent defined by cortical interactions with the actin cytoskeleton. In a polarized cell, there is it the potential for a positive feedback loop whereby actin and microtubule polarity stimulate each other.

Additionally, some microtubule stabilisers act directly on actin dynamics and vice versa. APC can both stabilize microtubules and nucleate actin filaments, with nucleation further stimulated by cooperation with the formin mDia1 [76, 94]. An additional role as a microtubule stabiliser has been reported for the formin mDia2, which is independent of its actin-nucleating activity [95]. Synergistic effects of such dual-acting proteins would be likely to be enhanced by the actin-microtubule crosslinking function of ACF7 [48] and Arg [66], which ensure a close proximity of both filament systems. Thus, crosstalk between the actin and microtubule cytoskeleton to

orchestrate cell polarity and migration is manifold and extends from dual-function molecules to complex feedback mechanisms in signalling networks.

9. Microtubule-dependent regulation of cell migration is impaired in cancers

While the coordination of cell migration is of crucial importance during embryonic development, wound healing and immune response, the deregulation of the migration machinery allows tissue invasion and metastasis by cancer cells. Thus understanding the regulation of cell migration might open new avenues for therapeutic approaches. Interestingly, microtubule-stabilizing factors implicated in the control of cell motility, such as APC, Dlg1 and RASSF1A, act as tumour suppressors and their loss is frequently associated with human cancers (see [96-98]). Moreover, non-specific microtubule stabilization by Taxol does inhibit cancer cell migration [99]. These observations suggest that a loss of microtubule-associated proteins and subsequent alteration of interphase microtubule dynamics stimulates uncontrolled motility in cancer cells that is associated with invasiveness and poor prognosis in cancer patients.

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