Scared stiff

Stabilizing the actin cytoskeleton to stop invading cancer cells in their tracks

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In recent years the concept of plasticity between invasion modes used by individual cancer cells has been gaining increasing interest in the field. Individually invading tumour cells can be divided into those that use a mesenchymal invasion mode, those that use “amoeboid” invasion and those that can switch between the two modes. The morphological distinctions between these different modes of invasion suggest that the actin cytoskeleton is likely to be a major contributor to the plasticity of cancer cell invasion mechanisms. We have recently investigated this idea by manipulating expression of Tm5NM1, one isoform of the tropomyosin family of actin-associating proteins. In a novel finding, we discovered that stabilizing the actin cytoskeleton via elevated expression of Tm5NM1 specifically inhibits mesenchymal-type cancer cell invasion, without causing transition to “amoeboid” motility—thus stopping the invading cancer cells in their tracks. The present perspective discusses our recent data in the context of current understanding of invasion plasticity and considers how stabilizing actin filaments may inhibit the mesenchymal invasion mode.

One of the striking features of invading cancer cells is the characteristic morphologies that they adopt, hinting at the distinct organization of actin filaments underpinning each invasion mode. When confronted with dense, fibrous collagen matrices some cancer cells adopt an elongated, polarized shape that characterizes

the mesenchymal mode of invasion. Conversely, in environments in which the pore size of the collagen matrix is permissive, some cells adopt the rounded, blebbing morphology of the ‘amoeboid’ invasion mode (inverted commas are used to indicate that this is not the same motility mechanism employed by amoebae).¹ The rounded appearance immediately suggests acto-myosin dependent contractility of the cortex and indeed ‘amoeboid’ invasion is dependent on acto-myosin contractility. Tension exerted through the contractile acto-myosin cytoskeleton at the cell cortex causes cell rounding and non-apoptotic membrane blebbing that promotes ‘amoeboid’ invasion¹ (Fig. 1A). In contrast, during mesenchymal invasion cells extend long polarized protrusions in the direction of cell migration and adopt elongated cell shapes. In this case, acto-myosin activity is critical for the retraction of the trailing edge during mesenchymal invasion and the contractile actin filaments are localized to the rear of the cell. At the other end of the cell, extension of the leading edge requires the polymerization of new actin filaments for the generation of protrusive force (Fig. 1B). The different organization of actin filaments with different properties (contractile versus protrusive) between “amoeboid” and mesenchymal cells therefore suggests that molecules that specify the function of actin filaments are likely to modulate the cell's invasion mode. Supporting this idea, we recently showed that overexpression of the actin-associating protein tropomyosin Tm5NM1 inhibits mesenchymal cell

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Figure 1. Tm5NM1 and invasion plasticity. (A) ‘Amoeboid’ invasion is characterized by reduced requirement for integrin-mediated adhesion to the extra cellular matrix. In this mode of invasion, tension derived through the contractile actin cytoskeleton at the cell cortex causes the fracture of the plasma membrane from the actin cortex, leading to bleb formation. Continued acto-myosin contractility leads to expansion of the bleb and invasion through the 3D matrix. This mode of invasion is independent of matrix metalloprotease activity and is stimulated by signaling downstream of Rho kinase. (B) In the mesenchymal mode of invasion, leading membrane protrusions are coordinated through actin polymerization and the formation of integrin-mediated adhesions with the surrounding matrix. In the trailing tail of the mesenchymal cell, the contractile actin cytoskeleton provides the necessary force to disassemble mature adhesions with the surrounding matrix, thereby allowing the cell to move forward. This mechanism is dependent on the secretion of matrix metalloproteases to create space for cell movement and is stimulated via signaling downstream of Rac GTPase. Cells that are competent to undergo mesenchymal to amoeboid transition (MAT) can be stimulated to transition between these two modes via manipulation of the relevant signaling pathways. (C) Elevated expression of tropomyosin isoform Tm5NM1 causes mesenchymal cells to undergo a morphological change, with cells rounding up and displaying multiple actin-rich pericellular spikes. Based on data from 2D culture where Tm5NM1 induces a switch to a predominance of fibrillar adhesions and ventral stress fibres, we hypothesize that the actin in the pericellular spikes may be firmly attached to the surrounding matrix via 3D fibrillar adhesions (putative integrin adhesions). Increased attachment of the actin filaments via transmembrane integrin receptors may thus inhibit the separation of the actin cytoskeleton from the plasma membrane that is required for the production of membrane blebs. Cells expressing exogenous Tm5NM1 undergo MAT following exposure to protease inhibitors.

invasion.² The tropomyosins are a multi-isoform family of actin-associating proteins, forming dimers that assemble as head-to-tail polymers that lay along the major groove of the actin filament. The likely mode of action of these non-muscle/cytoskeletal tropomyosins is to regulate the accessibility of the actin filament to other actin-regulatory proteins. For example, Tm5NM1 increases the association of activated myosin II motors with actin filaments.³ Based on this idea that the tropomyosins specialise the function of the associated actin filament, it is not surprising that the tropomyosins have emerged as regulators of cell migration (reviewed in ref. 4).

Our recent findings raise an interesting puzzle. A prime feature of ‘amoeboid’ invasion is the dependence on acto-myosin contractility. However, while Tm5NM1 induces myosin II recruitment to actin stress fibres and activation⁵ this is not accompanied by mesenchymal to amoeboid transition (MAT) in MAT-competent cells² (Fig. 1C). MAT can be induced by a number of mechanisms, including the inhibition of matrix metalloproteases,⁶ inhibition of Src kinase activity⁷ and the inhibition of Rac GTPase signaling.⁸ Unexpectedly, we established that Src kinase activity is suppressed following elevated Tm5NM1 expression yet this is not accompanied

by MAT.² Moreover, our earlier work showed increased focal adhesion stability in the presence of elevated Tm5NM1 and increased formation of Rac-dependent pre-cursor adhesion complexes in the absence of Tm5NM1 expression.⁹ These lines of evidence suggest that Tm5NM1 most likely also suppresses Rac GTPase activity. Yet, despite increased myosin II activity and signaling profiles that are generally associated with MAT, cells expressing elevated Tm5NM1 did not undergo MAT. These data suggest that Tm5NM1 suppression of the mesenchymal invasion mode is not simply due to suppression of the known MAT-stimulating signaling pathways. Secondly, these data implicate a critical role for the dynamics of the actin cytoskeleton in the conversion to amoeboid invasion that appears to override the signaling effects.

The contractile acto-myosin cytoskeleton is an essential regulator of the membrane blebs that form during ‘amoeboid’ cell invasion. Blebs nucleate in regions where cortical actin is detached from the plasma membrane and acto-myosin tension at the cortex then leads to increased hydrostatic pressure and expansion of the bleb. Following reassembly of the cortical actin cytoskeleton in the protruding bleb, the bleb then retracts.¹⁰ MAT-competent HT1080 fibrosarcoma cells grown in 3D collagen gels and expressing exogenous Tm5NM1 indeed lose the elongated mesenchymal phenotype and take on a rounded, shortened appearance. However, this change in cell phenotype is not accompanied by membrane blebbing, suggesting that the effects of Tm5NM1 on acto-myosin contractility are separable from the acto-myosin regulation of bleb initiation. Indeed, when MAT-competent cells expressing exogenous Tm5NM1 were treated with protease inhibitors these cells underwent MAT² (Fig. 1). Our data suggest the possibility that Tm5NM1 stimulated increases in acto-myosin activity that causes cell rounding is separable from the activity that is required to disrupt the interaction between the cortical actin cytoskeleton and the plasma membrane required for bleb initiation. Recent theoretical discussions suggest that under certain concentrations of actin-binding proteins the cortical cytoskeleton may achieve a

“metastable” state which can withstand small fluctuations that may otherwise lead to rupture, but is still susceptible to large fluctuations that lead to spontaneous fracture.¹¹ Potentially, Tm5NM1 may lead to a metastable cortical cytoskeleton that can be overcome, for example via the use of protease inhibitors.

We propose that one of the keys to how Tm5NM1 inhibits mesenchymal invasion without causing MAT may lie in the unique actin filaments and integrin-mediated focal adhesions that are stimulated by this tropomyosin isoform. We have previously shown that the Tm5NM1 tropomyosin isoform transitions cells to a phenotype of increased ventral stress fibres with associated fibrillar adhesions^{9,12} and this is an isoform specific effect.¹³ It is important to note that ventral stress fibres have been defined in 2D culture models¹⁴ and the form these actin filaments take in 3D environments remains to be determined. In 3D collagen gels cells expressing elevated Tm5NM1 display extensive pericellular filopodial-like actin rich structures² and the potential relationship between these structures and ventral stress fibres remains to be determined. The key point, however, is that at least in 2D the ventral fibres are associated with fibrillar adhesions that are transmembrane integrin-based adhesion sites. The fibrillar adhesions lie along the length of the ventral stress fibres and bring the filaments in close approximation to the plasma membrane and the underlying fibronectin matrix, indeed the fibrillar adhesions are part of the mechanosensory circuitry involved in the formation of fibronectin fibrils.¹⁵ Since it has been suggested that bleb nucleation depends on the strength of cortex-membrane attachments¹⁰ one possibility is that the specialized fibrillar adhesion sites may provide multiple anchorage points for the actin cytoskeleton to the cell membrane. Such adhesions may thereby protect against small fluctuations that could lead to rupture of the actin filaments

from the membrane that are necessary for membrane bleb initiation. Alternatively, as the fibrillar adhesions transmit tension to the surrounding matrix, the establishment of these specialized adhesion sites may reduce the acto-myosin contractile force below the critical threshold required for contractility-mediated rupture at the cortex, by instead transmitting the force to the surrounding matrix. In light of these suggestions, it is tantalizing that protease inhibitor treatment to induce MAT is accompanied by reduced cellular adhesion⁷ as this may provide an explanation for why protease inhibitor treatment of cells with elevated Tm5NM1 caused MAT. That is, the protease treatment may produce the necessary loss of adhesion that in turn promotes a break in the connection between the actin cytoskeleton and the plasma membrane required for blebbing and ‘amoeboid’ invasion.

Important outstanding questions now are to unravel the mechanism by which Tm5NM1 leads to the transition to the fibrillar adhesion/ventral stress fibre phenotype and whether indeed Tm5NM1 specifically effects the contractile acto-myosin cytoskeleton without causing cortex rupture and membrane blebbing. Such investigations will be critical to understanding how Tm5NM1 separates effects on ‘amoeboid’ invasion signaling pathways from the induction of a MAT. An important goal in the design of therapies to treat invasive, metastatic cancer is to revert the invasive phenotype. Our study has both revealed a novel mechanism to arrest cancer cell invasion without causing cells to switch to amoeboid invasion and highlighted a new role for regulators of actin filament specification in cell invasion plasticity.

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