

MT1-MMP: Endosomal delivery drives breast cancer metastasis

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The membrane-tethered membrane type 1–matrix metalloproteinase (MT1-MMP) mediates proteolysis-based invasive tumor growth. In this issue, Marchesin et al. (2015. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201506002>) describe a tug-of-war mechanism regulating dynein and kinesin motors to drive endosome tubulation and MT1-MMP delivery to the surface of cancer cells, identifying a crucial regulatory axis for tumor metastasis.

In metastasis, cancer cells escape from the primary tumor and disseminate in the body. To perform such an invasion, cancer cells must navigate within the meshwork of the ECM and cross tissue barriers, such as when entering and exiting the blood stream. Depending on the local density of the ECM, cells use either proteolysis-dependent or -independent migration modes (Wolf and Friedl, 2011). In proteolysis-based invasion, numerous studies point to the membrane-tethered membrane type 1–matrix metalloproteinase (MT1-MMP) as a key enzyme in the regulation of localized ECM breakdown (Itoh, 2015).

MT1-MMP contains a transmembrane domain and can thus be embedded in the plasma membrane of cancer cells (Itoh, 2015). Once exposed on the surface, MT1-MMP can also be reinternalized (Remacle et al., 2003), initiating a complex cycle of intracellular trafficking that results in either degradation of the proteinase or recycling back to the cell surface. As only surface-exposed MT1-MMP can contact ECM material, cancer cells must spatiotemporally adjust their levels of surface-localized MT1-MMP, depending on the pericellular environment. Invadopodia—ECM-degrading protrusions of cancer cells—are important sites of local MT1-MMP accumulation (Linder et al., 2011; Murphy and Courtneidge, 2011), and, thus, the molecular details of MT1-MMP delivery to invadopodia are the focus of intensive research efforts. Identification of pathways regulating MT1-MMP delivery is of fundamental interest to both cell biologists and clinicians interested in identifying prognostic markers of cancer progression or developing therapies targeted against metastatic cells.

In this issue, Marchesin et al. (2015) describe a mechanism for the localized delivery of MT1-MMP from endosomes to the surface of invadopodia in breast cancer cells, promoting invasiveness. Moreover, this study reveals a novel set of potential prognostic markers for aggressive breast cancer. As the GTPase ARF6 was previously associated with tumor invasion and metastasis, the researchers analyzed its contribution to MT1-MMP trafficking. siRNA-mediated knockdown of ARF6

or its effectors JNK interactor protein 3 and 4 (JIP3 and JIP4) in MT1-MMP-expressing breast cancer cells reduced MT1-MMP exocytosis and tumor cell invasion. Prior work showed that ARF6–JIP3/JIP4 and motor proteins associate on endosomes (Montagnac et al., 2009), so the authors postulated that these proteins regulate MT1-MMP-positive endosome movement. Depletion of ARF6 or JIP3/JIP4 indeed impaired endosome positioning, and image analysis of endosome position combined with ARF6 or JIP3/JIP4 silencing revealed that endosomes are docked at invadopodia through membrane-localized ARF6 associated with JIP3/JIP4. Searching for the motors contributing to endosome docking and movement, the researchers depleted crucial subunits of various motors, including p150^{Glued} (dynein–dynactin), KIF5B (kinesin-1), or KIF3A (kinesin-2), and observed that lack of any of these motors prevented normal MT1-MMP-positive endosome motility. Immunofluorescence analysis as well as coimmunoprecipitations confirmed the interaction between MT1-MMP and each of the three motor proteins. Interestingly, silencing of JIP3/JIP4 affected the association of MT1-MMP with kinesin-1/KIF5B and dynein–dynactin–p150^{Glued} but not with kinesin-2/KIF3A, suggesting that JIP3/JIP4 controls the transport of MT1-MMP endosomes through the association of kinesin-1/KIF5B and dynein–dynactin with these endosomes while having no effect on kinesin-2/KIF3A recruitment.

In addition, MT1-MMP exocytosis is known to involve the formation of tubular connections between endosomes and the plasma membrane in association with ECM fibers. To investigate the contribution of ARF6, JIP3/JIP4, and motor proteins to MT1-MMP-containing endosome exocytosis, Marchesin et al. (2015) used time-lapse microscopy in cells overexpressing or silenced for p150^{Glued}, KIF5B, or KIF3A. These experiments showed that ARF6, JIP3/JIP4, the dynein–dynactin–p150^{Glued} complex, and kinesin-1/KIF5B are required for tubulogenesis from MT1-MMP-positive compartments. Furthermore, tubulation is known to require WASH (Wiskott–Aldrich syndrome protein and scar homologue), an activator of the Arp2/3 complex, and the researchers found via knockdown experiments that WASH fulfills a dual function by recruiting JIP3/JIP4 to endosomes and promoting the F-actin remodeling necessary for endosome tubulation.

Overall, the authors propose that activated ARF6, through JIP3/JIP4, keeps dynein–dynactin anchored in place on microtubules. As dynein–dynactin and kinesin-1 are motors with

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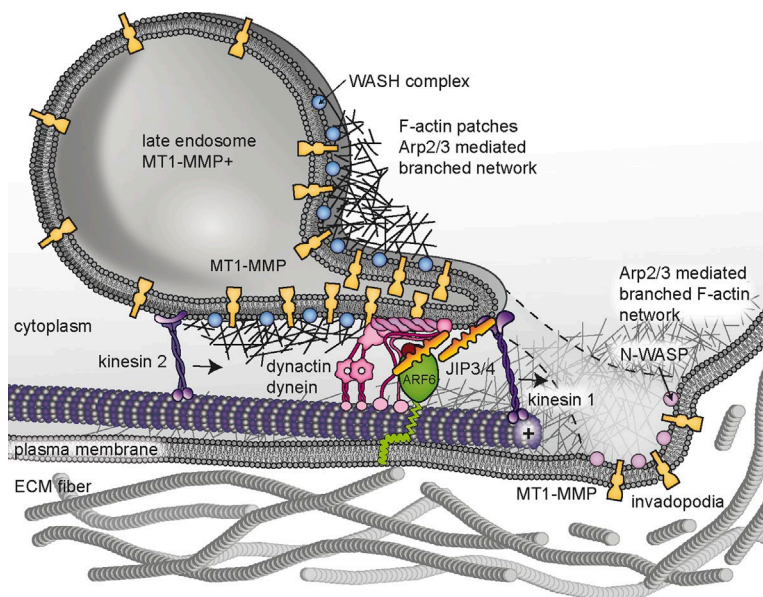


Figure 1. **Model of ARF6-JIP3/JIP4 function in MT1-MMP endosome movement.** ARF6 (green) lies at the plasma membrane and interacts through effectors JIP3/JIP4 (orange) with motors dynein-dynactin (pink) and kinesin-1 (purple). This complex controls the positioning and tubulation of MT1-MMP (yellow)-positive endosomes and coordinates with WASH (blue) to deliver MT1-MMP to invadopodia (figure republished from Marchesin et al., 2015).

opposite directionality, anchored endosomes become partially tubulated. The endosome tubules, with MT1-MMP embedded in their membrane, are subsequently shuttled to the plasma membrane at invadopodia thanks to WASH-mediated cytoskeletal remodeling. Inactivation of ARF6 lastly releases dynein-dynactin, ending the tug of war with kinesin-1/KIF5B and allowing clearance of endosomes from the membrane (Fig. 1, republished from Marchesin et al., 2015). The importance of this multiplayer mechanism is underscored by a microarray-based immunohistochemistry analysis of invasive cancer specimens, revealing a correlated up-regulation of ARF6 and kinesin-1/KIF5B together with MT1-MMP in cells of highly invasive breast cancers.

This study integrates a variety of previous and novel findings regarding the regulation of MT1-MMP trafficking. The authors started off by building on their prior work implicating ARF6 in the motility and metastatic potential of cancer cells (D'Souza-Schorey and Chavrier, 2006). In addition, the involvement of known ARF6 effectors JIP3/JIP4 in exosome movement by binding to kinesin-1 and dynein-dynactin (Bowman et al., 2000; Montagnac et al., 2009), as well as work showing that the microtubule-based activity of dynein, kinesin-1, and kinesin-2 drives MT1-MMP-containing vesicle delivery to the cell surface in macrophages (Wiesner et al., 2010), guided the researchers in their identification of the motor proteins mediating the effects of ARF6-JIP3/JIP4 on endosome movement. Similarly, the authors' model drew inspiration from the description of late endosomes exhibiting bidirectional mobility as a result of a tug of war between dynein and kinesin motors (Granger et al., 2014). Lastly, the authors confirmed that delivery of MT1-MMP to invadopodia requires WASH-induced tubular membrane connections between MT1-MMP endosomes and the invadopodial plasma membrane (Monteiro et al., 2013). However, the current study is the first to provide an integrated view of how the gears of several molecular machineries interlock to ensure localized delivery of MT1-MMP to invadopodia. ARF6 and JIP3/JIP4 especially emerge as crucial hubs that regulate the recruitment of molecular motors for the generation of MT1-MMP-containing endosomal tubules.

The model presented by Marchesin et al. (2015) provides a comprehensive analysis of localized MT1-MMP docking and

exocytosis and suggests further lines of research. For example, other regulators of MT1-MMP transport and membrane docking have been identified, such as the RabGTPases Rab5a and Rab4, which form a recycling circuitry for MT1-MMP in breast cancer cells (Frittoli et al., 2014); the SNARE protein VAMP7, which mediates docking of MT1-MMP vesicles (Steffen et al., 2008); cortactin, which recruits the membrane tubulating GTPase dynamin-2 to MT1-MMP-positive vesicles (Rossé et al., 2014); and the exocyst complex, which acts in concert with WASH to regulate delivery of MT1-MMP to the plasma membrane (Monteiro et al., 2013). How are all of these players organized in time and space to ensure coordinated delivery of MT1-MMP to invadopodia?

Also, the roles of kinesin-2 and microtubules in MT1-MMP exocytosis merit closer inspection. The authors show that kinesin-2 drives delivery of MT1-MMP vesicles to the cell periphery, similar to what has been shown in macrophages (Wiesner et al., 2010). However, in contrast to kinesin-1, kinesin-2 is not bound by JIP3/JIP4 and, therefore, does not induce endosome tubulation. Consistently, in the microarray analysis performed by Marchesin et al. (2015), kinesin-2 clusters away from ARF6, JIP3/JIP4, and kinesin-1. The importance of kinesin-2 in MT1-MMP trafficking in cancer cells is thus currently unclear. Furthermore, the molecular hub between MT1-MMP vesicles, WASH/Arp2/3-generated actin networks, and microtubules is surely a treasure trove of MT1-MMP-relevant regulators waiting to be discovered. For example, IQGAP regulates MT1-MMP exocytosis by binding to the exocyst (Sakurai-Yageta et al., 2008), with the exocyst also binding to WASH on MT1-MMP-containing endosomes (Monteiro et al., 2013). As IQGAP interacts with the microtubule plus tip protein CLIP-170 as well (Fukata et al., 2002), it may serve to attach microtubules to the MT1-MMP docking site. Other crucial regulators are sure to emerge from future work studying the interaction of microtubules and the actin cytoskeleton in the context of MT1-MMP vesicle docking. Moreover, MT1-MMP delivery might also be regulated by proteins that were not previously linked to endosome exocytosis. One way to identify such new regulators is to turn the approach used in this study on its head by detecting proteins with altered expression profiles in cancers and

then fitting them into the MT1-MMP circuitry. A comparable approach, based on protein expression levels and tissue analysis, has recently been undertaken for adenocarcinoma cells and yielded the identification of GATA binding protein 3 as a novel marker of aggressive adenocarcinomas (French et al., 2015).

The identified ARF6–JIP–motor protein axis is clearly of major importance for breast cancer invasiveness. However, cancer cells of different origins may use other strategies for MT1-MMP trafficking, exocytosis, or recycling. Considering that the recycling circuitries for MT1-MMP in breast cancer cells and other cell types such as primary macrophages are remarkably different (Wiesner et al., 2013; Frittoli et al., 2014), this should be worthy of further investigation. Similarly, all cancer cells may not regulate MT1-MMP activity in the same manner. Indeed, delivery of MT1-MMP does not necessarily imply activation of the protease. MT1-MMP-dependent proteolysis is regulated on multiple levels, such as by removal of a prodomain that masks the catalytic center, oligomerization, interaction with inhibitors, or shedding of the active part of the molecule at the cell surface (Itoh, 2015). Melanoma cells regulate proteolytic activation of MT1-MMP by controlling its association with furin in a post-Golgi compartment (Mazzone et al., 2004), indicating that the inhibitory prodomain can be removed before MT1-MMP insertion into the membrane. Still, it is unclear whether all cancer cells follow a similar strategy.

Elucidating the molecular details of MT1-MMP trafficking and delivery in various cancer subtypes is essential, as it could point to cell type-specific markers of tumor aggressiveness and lead to the development of treatments targeting invasive cells. However, the challenge remains: How can the treatment be tailored to specifically target metastasis and spare noncancerous cells? Cells use their molecular toolbox for multiple purposes, and the mediators of MT1-MMP trafficking, docking, and exocytosis therefore also play broader roles in cellular trafficking and other processes, indicating that a therapeutic against such candidates could have side effects on normal cells. It is thus crucial to identify a molecular “fingerprint” that is sufficiently specific to aggressive tumor cells to target MT1-MMP regulation only in these cells. By showing a correlation between the levels of ARF6, MT1-MMP, kinesin-1/KIF5B, and breast cancer invasiveness and determining their exact contribution in tumor cells, the work of Marchesin et al. (2015) opens the door to defining a new set of prognostic markers for aggressive breast cancer and takes an important step toward determining the specific molecular signature of aggressive breast cancer.

Acknowledgments

The author apologizes to those whose work was not mentioned owing to space limitations. The support of Deutsche Forschungsgemeinschaft (grants LI925/2-2 and LI925/3-2) and of Wilhelm Sander-Stiftung (grant 2014.135.1) is gratefully acknowledged.

Submitted: 2 October 2015

Accepted: 2 October 2015

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