Trends in Cancer

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Review

Engine shutdown: migrastatic strategies and prevention of metastases

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Most cancer-related deaths among patients with solid tumors are caused by metastases. Migrastatic strategies represent a unique therapeutic approach to prevent all forms of cancer cell migration and invasion. Because the migration machinery has been shown to promote metastatic dissemination, successful migrastatic therapy may reduce the need for high-dose cytotoxic therapies that are currently used to prevent the risk of metastatic dissemination. In this review we focus on anti-invasive and antimetastatic strategies that hold promise for the treatment of solid tumors. The best targets for migrastatic therapy would be those that are required by all forms of motility, such as ATP availability, mitochondrial metabolism, and cytoskeletal dynamics and cell contractility.

The importance of cell migration during metastatic cascade as a basis for migrastatic therapy

Tumor invasion and metastasis are ground-breaking events that transform a localized primary tumor into a severe systemic and life-threatening disease. Consequently, most cancer-related deaths among patients with solid tumors are caused by metastases. Because the migration machinery has been shown to promote metastatic dissemination, there is a strong correlation between the molecular mechanisms of cell migration and the progression of metastatic disease [1,2].

Cell migration can be classified into single-cell migration modes (mesenchymal, amoeboid, osmotic engine) and collective migration modes (Figure 1). Each mechanism of cell migration requires energy that is consumed by a different set of molecules for conversion to mechanical power [3]. Migration modes are closely associated with the structure and molecular composition of the extracellular matrix (ECM), cellular energy status [4–6], the characteristic structure of the cytoskeleton, and the use of specific integrins, matrix-degrading enzymes, cell–cell adhesion molecules, and signaling pathways. In response to the gain or loss of these key molecular determinants, cells can flexibly modify their shape and migration mechanism [7,8]. Migrastatic strategies represent a unique therapeutic approach to prevent all forms of cancer cell migration and invasion through the ECM. A successful migrastatic therapy may reduce the need for high-dose cytotoxic therapies that are currently used to prevent the risk of metastatic dissemination. The term 'migrastatics' is used here for drugs interfering with cancer cell migration or invasion.

Invasive cells use diverse strategies to invade through ECM barriers. Nevertheless, there are some important similarities. All known strategies need to increase ATP production within invading cells (enhanced glucose uptake, creatine–phosphagen system, specific mitochondria localization) [6,9–11]. This ATP must be converted to mechanical power through cytoskeletal dynamics, cell contractility, or osmotic engine mechanisms. The importance of ATP reserves during migration is evidenced by the fact that extracellular ATP can enhance the motility and invasion of cancer cells [12]. Cells also usually migrate in the direction of least confinement to minimize energy costs.

Highlights

Metastases are the most common cause of cancer-related death. Although cancer survival rates have significantly improved over the years owing to better diagnostic processes and cytotoxic therapies, novel approaches targeting metastases are still needed.

Because the migration machinery has been shown to promote metastatic dissemination, a successful migrastatic therapy administered simultaneously with standard therapy could show a synergistic effect and therefore may reduce the need for aggressive high-dose cytotoxic therapies that are currently used to prevent the risk of metastatic dissemination.

The major advantage of migrastatic therapy is that selection pressures caused by this type of therapy may not cause resistance to conventional therapies based on antiproliferative or cytotoxic effects because the mechanisms targeted are of a completely different nature.

The best targets for migrastatic therapy are probably those that are required by all forms of motility, such as ATP availability, mitochondrial metabolism, cytoskeletal dynamics, and cell contractility.

An increase in energy consumption is needed for migration through more demanding environments, and changes in intracellular ATP/ADP levels are directly tied to changes in cell migration speed. Mitochondria-targeting strategies causing ATP depletion can be complemented by approaches that force cells to use more energy-consuming types of movement or that prevent them from moving in less energy-consuming ways.

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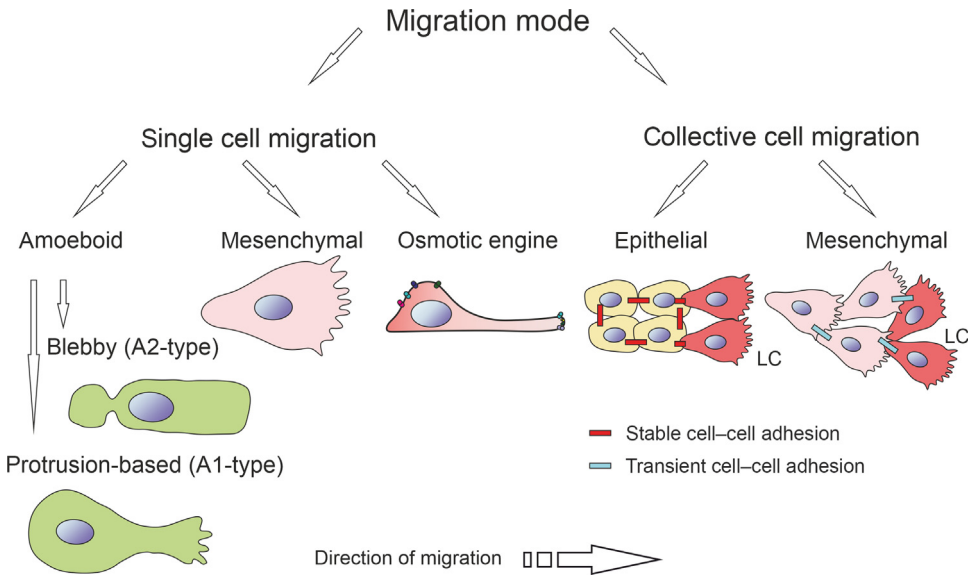

Figure 1. Cancer cell migration can be classified into single-cell and collective migration modes. Amoeboid migration is characterized by low adhesion forces and uses either a high-contractility myosin II-dependent mode driven by membrane blebbing (A2 type) or a protrusion-based mode (A1 type) that occurs under low cell contractility. Mesenchymal cell migration is characterized by high cell polarization, and in the case of spaces in the extracellular matrix (ECM) that are too small for cell passage, by proteolytic ECM remodeling by matrix metalloproteases and serine proteases. The osmotic engine model predicts that migration can occur regardless of actomyosin contractility through a process of polarized uptake and expulsion of water. Epithelial collective cell migration involves cohesive groups of cells maintaining stable cell–cell connections. Partial epithelial–mesenchymal transition (EMT) in the leader cells (LCs; depicted in red) can preserve stable cell–cell adhesion and allow epithelial sheet migration. Mesenchymal cells or cells after full EMT (functional transition of polarized epithelial cells into mobile mesenchymal cells) migrate directionally as a collective, but only form transient cell–cell adhesions. The motility of individual cells is increased but LCs are also needed for mesenchymal collective migration to maintain the stable direction of movement.

The energy needed to generate force for ECM displacement increases with the degree of spatial confinement and with increasing cell or ECM resistance to deformation (stiffness) [5]. Agents that can increase cellular stiffness in some cancer cells include, for example, cisplatin or docetaxel [13]. The low energy levels probably also disrupt the collective cell migration mode because of the lack of leader cells (LCs) [4]. Consequently, the best targets for migrastatic therapy would be those that are required by all forms of motility, such as ATP availability, mitochondrial metabolism, cytoskeletal dynamics, and cell contractility.

In this review we focus on anti-invasive and antimetastatic strategies that show promise in the treatment of solid tumors. We explain why migrastatic strategies must not be focused on mechanisms occurring in only one mode of migration and that the migratory plasticity of cancer cells must also be considered. We also discuss potential universal targets that hold promise for migrastatic therapy.

Targeting the mechanisms involved in the mesenchymal type of migration may not be sufficient

Mesenchymal cell migration is accompanied by high cell polarization that generates a leading edge with actin-rich protrusions that allow adhesive interactions followed by contractile retraction of the cell rear. This motility process depends on regulators such as Cdc42 (cell division control protein 42 homolog), Rac1, ROCK (Rho-associated protein kinase), and RhoA [14]. The main

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forces driving the cell forward are actin polymerization and actomyosin contractility in cooperation with integrins β1 or αvβ3 [15,16]. To migrate through the complex ECM, two main strategies can be adopted by cancer cells: ECM degradation or shape deformation that allows cells to squeeze through narrow gaps in ECM [15]. The rate-limiting role in migration is played by the nucleus, which is the stiffest cellular component. In collagen gels, tumor cell arrest occurs at pore sizes smaller than ~7 μm^2, which corresponds to ~10% of the nuclear cross-sectional area. Below this pore size threshold, the cells cannot migrate in the absence of ECM remodeling [17]. However, knockdown of lamin A, one of the key components of the nuclear envelope, can decrease nuclear stiffness and enhance the migration of cancer cells through pores smaller than ~7 μm^2. However, these cells can be more prone to apoptosis [18].

For these reasons, therapeutic targeting of the enzymes that remodel the ECM, such as matrix metalloproteases (MMPs) or serine proteases, seems to be reasonable. Surprisingly, inhibition of these enzymes did not prevent tumor cell invasion and showed weak benefit in some animal cancer models as well as in clinical trials in humans [19–21]. The hydroxamate-type MMP inhibitor batimastat even promoted liver metastasis in mice [22]. Phase 3 clinical trials using marimastat, prinomastat (AG3340), and BAY 12-9566 alone or in combination with standard chemotherapy in patients with advanced cancers (lung, prostate, pancreas, brain, gastrointestinal tract) showed no clinical efficacy [21]. The failure of MMP inhibitors can be explained for example by modes of cellular migration that are independent of structural matrix remodeling (amoeboid migration could be even triggered by MMP inhibition) or by the presence of the pre-existing physiological microtracks in the ECM that enable MMP-independent migration.

Cell migration through preformed ECM microtracks may be effectively inhibited by targeting cytoskeletal dynamics because this type of migration is driven by polarized protrusions and elongations at the leading edge which are mediated by actin polymerization and enhanced by the microtubules [23]. Interestingly, actomyosin contractility, but not traction generation, was needed for effective microtrack migration. Microtrack migration exhibited elements of both mesenchymal and amoeboid motility because cells adapted their migration mode after integrin blocking and cell contractility inhibition (cells exhibited stable mesenchymal-to-amoeboid transition following treatment with integrin and MMP inhibitors in a 3D matrix) [23]. Furthermore, inhibitors of β1-integrins, myosin, Rho, or ROCK do not impair confined migration through channels 3 μm in width even though these treatments repress unconfined migration. Confined migration persists even when F-actin is disrupted, but in this case depends largely on microtubule dynamics [24]. It was shown that microtubules can act as proprioceptive sensors that control cell shape and actomyosin retraction to sustain cellular cohesion during amoeboid migration [25]. Thus, amoeboid migration appears to involve mechanisms that bypass tissue barriers independently of ECM degradation. Amoeboid single-cell migration can also result from collective cell migration after treatment with adhesion-disrupting agents such as anti-β1-integrin antibodies or cadherin antagonists. By interfering with cell–cell junctions, as well as adhesion to collagen fibers, single cells detach and migrate by β1-integrin-independent amoeboid mechanisms. Similar transitions might also occur spontaneously during cancer progression [26]. However, the failure of agents targeting the mechanisms of mesenchymal migration should not be considered a final defeat. These agents can probably be used in combination with agents targeting other types of migration. These combinatorial approaches based on synergistic effects may subsequently lead to a reduction in the migratory plasticity of cancer cells.

Targeting the migratory plasticity of cancer cells

The mesenchymal-to-amoeboid transition is a manifestation of migratory plasticity that not only promotes tumor cell dissemination but also helps tumor cells to escape medical treatment.

However, some molecules, such as NEDD9, seem to be needed for both types of movement. NEDD9 seems to be necessary for both mesenchymal and amoeboid individual cell migration/invasion in triple-negative breast cancer. NEDD9 deficiency results in the acquisition of an amoeboid morphology but severely limits all types of cell motility. Simultaneous depletion of NEDD9 or inhibition of aurora kinase A in combination with inhibition of the amoeboid driver ROCK resulted in an additional decrease in cancer cell migration/invasion [27]. Combined blockade of extracellular proteases and ROCK negates the ability of tumor cells to switch between modes of motility and synergizes to prevent tumor cell invasion [28]. Nevertheless, ROCK inhibitors should be used with caution. Indeed, Rho/ROCK inhibitors such as Y27632 and fasudil (HA-1077) decreased the migration and invasion capacities of several cancer cell types [29–32]. However, it was also shown that Y27632, which impairs amoeboid-like invasion, restores cell-surface expression of α2β1-integrin, enhances focal adhesion kinase (FAK) autophosphorylation, and therefore can promote mesenchymal invasion [32–36]. In these cases, FAK inhibitors and/or FAK displacement from focal adhesions can be useful [37,38]. Furthermore, ROCK inhibition promoted NaV1.5 sodium channel-dependent SW620 colon cancer cell invasiveness [39], and ROCK1 signaling has been implicated in the regulation of Beclin1-mediated autophagy during metabolic stress, where inhibition of ROCK1 activity increases Beclin1–Bcl-2 association, thus reducing autophagy [40].

KD-025 (or SLx-2119) is a novel selective ROCK2 inhibitor that is 200-fold more selective for ROCK2 than ROCK1. KD-025 enhances the efficacy of conventional chemotherapeutic drugs in ABCG2-overexpressing leukemia cells by restraining the efflux activity of ABCG2 and obstructing ATPase activity [41]. However, ROCK2 inhibition triggered the initial induction of LC formation and induced collective invasion from cysts in colorectal cancer [42]. Moreover, the presence of ROCK inhibitors reduced the actomyosin contractility required for entotic cell death, resulting in promotion of tumor growth in some types of cancer [43]. Some hopes were raised by the ATP-competitive AKT kinase inhibitors AT13148 and CCT129254 that also inhibit the Rho kinases ROCK1 and ROCK2 because they impaired both amoeboid and mesenchymal modes of invasion in cell cultures [44]. CCT129254 was well tolerated in mice when dosed daily at up to 200 mg/kg [45]. Nevertheless, hypotension, pneumonitis, skin rash, and elevated liver enzymes were associated with AT13148 treatment. The narrow therapeutic index and the pharmacokinetic profile of AT13148 led to the recommendation not to develop this compound further [46].

Targeting the cytoskeleton

All three cytoskeletal networks – actin microfilaments, microtubules, and intermediate filaments – closely cooperate to control migration and they share some targetable regulatory proteins [47]. For example, Ser/Thr casein kinase CK2 is required for the proper assembly and function of microfilaments and microtubules, and is directly involved in the regulation of actin polymerization (through WASP proteins and formin) and its dynamics (through coronin, PACSIN-1, or CKIP-1). CK2 may also affect the structure and function of microtubules by direct phosphorylation of tubulin and a wide range of microtubule-associated proteins [48]. CK2 is a well-established therapeutic target in cancer, but only one small-molecule inhibitor has reached clinical trials – CX-4945 [49,50]. Another CK2 inhibitor, the indeno[1,2-b]indole derivative 5-isopropyl-4-methoxy-7-methyl-5,6,7,8-tetrahydroindeno[1,2-b]indole-9,10-dione (5a-2), was recently tested on cell lines. Whereas CX-4945 showed a strong proapoptotic effect on tumor cells, 5a-2 was more effective in inhibiting tumor cell migration. This can be explained by the different subcellular distributions of these compounds leading to different sites of CK2 inhibition. For 5a-2, 71% was detectable in the cytoplasm, whereas 49% of intracellular CX-4945 was localized in the nuclear fraction [50].

The actin cytoskeleton is indeed a potentially vulnerable feature of cancer cells, but the therapeutic potential of actin-targeting agents has been impeded by high toxicity because the tested anti-actin agents are unable to discriminate between the actin cytoskeleton of tumor cells and muscle actin filaments. Although actin-targeting drugs such as cytochalasin D and jasplakinolide have shown great promise, neither has made it to clinical trials. The possibility of targeting cancer-specific isoforms of tropomyosin, a core component of actin filaments, was tested in the study of Stehn *et al.* where compound TR100 inhibited tumor growth in melanoma and neuroblastoma mice models [51]. An effect on the actin cytoskeleton was also observed with the widely used cytostatic cisplatin [52]. This may be one of the reasons for the efficacy of cisplatin in preventing metastasis [53,54]. When administered at a higher dose (6 mg/kg), cisplatin inhibited both cancer growth and metastasis in a mouse model, but with strong side effects, whereas a lower dose (2 mg/kg) of cisplatin prevented cancer metastasis without visible cytotoxic effects [55]. Nevertheless, the tumor-suppressive actin-remodeling effect of cisplatin [13] is probably cancer type-dependent because cisplatin-treated melanoma cells exhibit a significant upregulation of FAK-and MAPK-mediated signaling that promotes the chemoresistance and invasiveness of these cells. Accordingly, cisplatin is not effective against melanoma [56,57].

As mentioned previously, confined migration can persist even when F-actin is disrupted, but this depends largely on microtubules [24]. Furthermore, the microtubule-targeting agent (MTA) paclitaxel is 100-fold more effective at blocking migration in a 3D matrix than on 2D matrices [58]. This fact demonstrates that cells use different migration modes depending on physical confinement. MTAs (paclitaxel, docetaxel, cabazitaxel, and vinca alkaloids) are widely used in the clinic but their toxicity is often dose-limiting. Interestingly, the migrastatic effects of MTAs have been observed at lower concentrations than those required for cytostatic effects. The subtoxic concentrations seem to be sufficient to inhibit cell migration [59–65]. Toxic side effects of MTAs can also be, at least partly, eliminated by synergy with other anticancer drugs. Such synergy was shown with compounds that target a specific population of actin filaments containing the cancer-associated tropomyosin Tpm3.1 (ATM-3507 and TR100) [66,67]. Other toxic side effects of MTAs can be eliminated, at least partially, by conjugation of the drugs with various carriers or nanoparticles.

Nanocarriers targeting migration

Nanocarriers can often improve the delivery of conventional therapeutic drugs. As previously stated, integrins are closely involved in cancer cell migration. Among inorganic nanomaterials, gold nanoparticles (AuNPs) functionalized by Arg-Gly-Asp peptide (RGD motif) were found to repress cell migration by targeting integrins. In particular, integrin αvβ3, that is highly overexpressed in cancer cells, can bind to the RGD motif, which makes it a promising target for nanotherapy either for tumor detection or for enhanced specificity of drug delivery [68]. Accordingly, molybdenum dioxide ($MoS_{2)}$/gadolinium (Gd) particles containing RGD sequences showed potential as contrast agents for magnetic resonance imaging [69]. Other effects of AuNPs include changes in the cytoskeletal networks of treated cells, especially a reduction of filopodia and lamellipodia. Furthermore, broad perturbations were observed in Rho GTPases, microtubule, actin, and kinase-regulated signaling pathways downstream of integrins [70]. This antimetastatic effect of AuNPs can be enhanced by activating AuNPs with near-IR light (808 nm) which generates heat that can be further used for photothermal therapy (PTT) [71]. AuNRs in combination with PTT were successfully used in the treatment of mammary gland tumors in dogs and cats. No metastasis or cancer relapse was observed 1 year after treatment [72]. Upon nuclear targeting of AuNPs by a nuclear localization signal, ovarian cancer cell motility decreased significantly due to overexpression of lamin A/C and enhanced nuclear stiffness [73]. Silicon dioxide (SiO_2),

titanium dioxide (TiO_2), and hydroxyapatite nanoparticles strengthened intracellular tension and retarded collective cell sheet migration of TR146 cells. This could be caused by destabilization of the microtubule network, leading to a drastic change in the magnitude and spatiotemporal distribution of cell traction [74]. In another study, high concentrations of iron oxide nanoparticles within cells affected the cytoskeleton and focal adhesion kinase [75].

Magnetic nanoparticles (MNPs), in particular those comprising polyethylenimine (PEI)-coated superparamagnetic iron oxide nanoparticles (SPIONs), showed their potential to reduce the expression of the cell migration-associated genes *MMP9* and *MMP14* and to alter actin polymerization. *In vivo* experiments conducted on MDA-MB-231 xenograft tumors in mice inhibited formation of blood vessels and increased macrophage infiltration, indicating the potential use of SPIONs as antiangiogenic agents [76]. Vascular endothelial growth factor (VEGF, a marker of angiogenesis), MMP2, and MMP9 were successfully downregulated by tangeretin-zinc oxide quantum dots (Tan-ZnO QDs). Tan-ZnO QDs studied on H358 metastatic lung cancer cells were shown to decrease cell migration and proliferation, promote G2/M cell-cycle arrest, and induce nuclear fragmentation and apoptosis [77]. In mice bearing 4T1 metastatic breast cancer tumors, downregulation of MMP2 and MMP9 activity and expression was observed by using nanosized lysolipid-containing thermosensitive liposomes transporting the MMP inhibitor marimastat. Antimetastatic effects of such liposomes were demonstrated by a reduction in lung metastatic nodules as well as a decrease in the number of tumor site microvessels [78]. Solid lipid nanoparticles (SLNs) are another group of lipid-based nanomaterials. Docetaxel-loaded SLNs (DTX–SLNs) were investigated in 4T1, MCF7, and NIH-3T3 cells, as well as in BALB/c mice bearing 4T1 tumors. In this study, DTX-SLNs were reported to induce microtubule damage and apoptosis, and decreased Bcl-2 and Ki-67 expression or IL-6 production, leading to lung metastasis prevention as well as overall inhibition of tumor progression [79].

Therapeutic drugs with repurposing potential for migrastatic therapy in clinical trials or animal studies are summarized in Table 1. Some of these drugs failed in clinical tests focused on cancer. However, tumor size or advanced metastatic disease is unlikely to be influenced by migrastatic therapy, and this can lead to the false assumption that a given therapy is ineffective.

ATP availability and mitochondrial metabolism influence cell migration

Both cytoskeletal reorganization and ECM remodeling and/or deformation require ATP-dependent processes such as actin polymerization and actomyosin contractility. Maintaining an adequate supply of ATP is crucial for cellular motility, and migrating cells thoroughly tune their energy utilization relative to the structure and mechanics of their microenvironment. An increase in energy consumption is needed for migration through more demanding environments, and changes in intracellular ATP/ADP levels are directly tied to changes in cell speed [80]. Collectively migrating cells migrate more effectively than single cells. The leading edge of the group is occupied by specialized front cells, the LCs [81]. Some evidence suggests that alternative metabolic pathways are activated in LCs to fuel the metastatic process [82]. LCs probably require more energy than follower cells because they generate the pushing and pulling forces that are necessary for migration through the ECM. The energy levels of LCs (as revealed by the intracellular ATP/ADP ratio) must be high to exceed a threshold for invasion. Forward movement of an LC gradually exhausts the available ATP reserves until the exhaustion reaches a threshold level under which it can no longer invade. The LC is then replaced by the follower cell with sufficient energy supplies. When placed in a denser collagen matrix, LC durability decreases [4]. Some studies also showed that functional mitochondrial oxidative phosphorylation (OXPHOS) is crucial for metastatic spreading. Pharmacological inhibition of pyruvate dehydrogenase (the enzyme linking glycolysis to the tricarboxylic acid cycle in mitochondria), removal of mitochondrial DNA, or dihydroorotate

Table 1. Therapeutic drugs with repurposing potential for migrastatic therapy[a,b]

Pathway	Targeted molecule	Compound	Status	Refs
Cell adhesion	αvβ3 and αvβ5 integrins	Cilengitide	Clinical trials (Phase 3; glioblastoma); NCT00689221	[145]
	Integrin α5β1	Volociximab	Clinical trials (Phase 2; lung cancer); NCT00278187	[146,147]
	Integrin α5β1	ATN-161	Clinical trials (Phase 1; malignant glioma); NCT00352313	[148]
	Focal adhesion kinase (FAK)	PF-00562271	Clinical trials (Phase 1; pancreatic, head and neck, and prostatic neoplasms); NCT00666926	[149,150]
	Focal adhesion kinase (FAK)	Defactinib	Clinical trials (Phase 2; metastatic melanoma); NCT04720417	[151]
	Focal adhesion kinase (FAK)	GSK2256098	Clinical trials (Phase 2; progressive meningioma); NCT02523014	[152,153]
	αvβ3, α3β1, and α5β1 integrins	Gold nanorods (AuNRs)	Animal studies	[70,73]
Cell polarization	Rac1 and Cdc42	*R*-enantiomer of ketorolac	Animal studies	[154]
	PI3K/mTOR	NVP-BEZ235	Clinical trials (Phase 1; breast cancer); NCT00620594	[155–157]
ECM remodeling	MMP	Marimastat	Clinical trials (Phase 3; lung cancer); NCT00003011	[21]
	MMP	Prinomastat	Clinical trials (Phase 3; lung cancer); NCT00004199	[21]
	MMP	BAY 12-9566	Clinical trials (Phase 3; advanced ovarian cancer); NCIC CTG	[21,158]
	MMP9 and MMP14	PEI-SPIONs	Animal studies	[76]
	MMP2 and MMP9	Lysolipid-containing thermosensitive liposomes	Animal studies	[78]
Actomyosin contractility	ROCK	Y27632	Animal studies	[32,159]
	ROCK	Fasudil	Animal studies; clinically approved for the treatment of cerebral vasospasm	[32,159,160]
	ROCK	H-1152	Animal studies	[161]
	ROCK	RKI-1447	Animal studies	[162]
	ROCK	Wf-536	Animal studies	[163]
	ROCK2	KD-025/SLx-2119	Clinical trials for psoriasis vulgaris and idiopathic pulmonary fibrosis (NCT02106195, NCT02317627, NCT02688647)	[41]
	ROCK/AKT	CCT129254	Animal studies	[44]
Assembly and function of microfilaments or microtubules	CK2	CX-4945	Clinical trials (Phase 1; cholangiocarcinoma); NCT02128282	[164]
	β-Tubulin	Paclitaxel	FDA-approved	[60]
	β-Tubulin	Docetaxel	FDA-approved	[60]
	β-Tubulin	Cabazitaxel	FDA-approved	[60]
	αβ-Tubulin dimer	Vinca alcaloids (vincristine, vinblastine, vinorelbine, vindesine, vinflunine)	FDA-approved	[60]
	β-Tubulin	Docetaxel-loaded SLNs	Animal studies	[79]

dehydrogenase (DHODH) inhibition reduced the ability of cancer cells to invade [82–84]. Commander *et al.* also defined a pyruvate dehydrogenase dependency of LCs that can be therapeutically exploited with mitochondria-targeting alexidine dihydrochloride [82].

Many studies showed that, in migrating cancer cells, mitochondria localize at the leading edge (along microtubules) to support enhanced cell motility and invasion by providing local sources of energy [85,86]. The leading edge is fueled by OXPHOS and mitochondrial ATP, rather than by glycolysis [86]. OXPHOS is also needed for mitochondria repositioning because pharmacological inhibition of mitochondrial complex I (by rotenone), complex III (by antimycin A), complex V (by oligomycin), or a mitochondrial uncoupler (carbonyl cyanide *m*-chlorophenyl hydrazine) inhibited mitochondrial repositioning to the cortical cytoskeleton. Gamitrinib, a mitochondrial-targeted Hsp90 (heat-shock protein 90) inhibitor that induces degradation of the mitochondrial complex II subunit SDHB (succinate dehydrogenase complex iron–sulfur subunit B), successfully prevented the accumulation of mitochondria in FAK-containing focal adhesions [87].

Energy demand and consequently AMPK (AMP-activated protein kinase) activity are elevated in the leading edge of the migrating cell. This localized activation of AMPK increases mitochondrial repositioning [86]. Mitochondrial Rho-GTPase 1 (Miro1), a mediator of microtubule-based mitochondrial motility, is involved in this process. Mitochondrial repositioning, focal adhesion assembly, and stability are decreased in *Miro1*$^{-/-}$ mouse embryonic fibroblasts. Consequently, *Miro1*$^{-/-}$ cells migrated more slowly during both collective and single-cell migration [88]. Cancer cells often reprogram mitochondrial dynamics managed by syntaphilin (SNPH), kinesin KIF5B, and GTPase Miro1/2 to localize mitochondria to the cortical cytoskeleton and power the machinery of cell motility [89]. High SNPH expression can reduce the velocity and distance covered by individual mitochondria by suppressing mitochondrial dynamics, and consequently may inhibit chemotaxis and metastasis. siRNA silencing of the anterograde kinesin KIF5B or Rho-GTPase Miro1 suppressed tumor cell invasion induced by loss of SNPH. By contrast, silencing of Miro2 did not reduce tumor cell invasion in SNPH knockdown cells [89]. The asymmetric distribution of mitochondria within migrating cells can be also disrupted by interfering with mitochondrial fusion (Opa1) or fission (Drp1) proteins. Such interference significantly reduces the number of cells with anterior localization of mitochondria, and significantly decreases the velocity and directional migration of the fastest moving cells [90]. Silencing of Drp1, overexpression of Mfn1 (fusion protein), or treatment with a mitochondrial uncoupling agent or ATP synthesis inhibitor reduced lamellipodia formation and decreased breast cancer cell migration and invasion, suggesting that mitochondria play a functional role in breast cancer metastasis [91]. Functional mitochondria also seem to be necessary for LC activity and collective migration [4]. Inhibition of mitochondrial ATP synthesis can influence all known types of migration because actomyosin contractility, actin treadmilling, and active solute pumping are driven by energy-consuming processes. Even an osmotic engine – that does not require ATP for actomyosin contractility or actin treadmilling – requires ATP as a direct activator of NHE1 and other solute carriers or ion channels [92]. Therefore, targeting mitochondria can be a rational strategy for the development of migrastatic and antimetastatic agents for cancer treatment.

Notes to Table 1:
[a]Abbreviations: AKT, Rac-α serine/threonine protein kinase; Cdc42, cell division control protein 42 homolog; CK2, Ser/Thr casein kinase; ECM, extracellular matrix; MMP, matrix metalloprotease; mTOR, mammalian target of rapamycin; NCIC CTG, National Cancer Institute of Canada Clinical Trials Group; PEI, polyethylenimine; PI3K, phosphoinositide 3-kinase; Rac1, Ras-related C3 botulinum toxin substrate 1; ROCK, Rho-associated protein kinase; SLNs, solid lipid nanoparticles; SPIONS, superparamagnetic iron oxide nanoparticles.
[b]Status: 'Clinical trials' means that the drug was involved in any type of clinical trial (not necessarily a clinical test for cancer). We only considered agents that have been tested in animals or in clinical trials because their future therapeutic potential is higher and their repurposing is easier. The references cited refer to the use of these drugs in cancer therapy.

Mitochondria-targeting drugs with potential for cancer therapy

Many mitochondria-targeting drugs with potential as cancer therapeutics target electron chain (ETC) transport complexes. This results in a drop in ATP production and consequent activation of AMPK and inhibition of mTORC1 (mammalian target of rapamycin complex 1). Rotenone is a potent inhibitor of mitochondrial complex I (CI) because it inhibits the transfer of electrons from iron–sulfur centers in CI to ubiquinone. Rotenone is highly lipophilic, easily crosses the blood–brain barrier, and accumulates in subcellular organelles including the mitochondria [93]. It also inhibits microtubule assembly [94] and restrains colon cancer cell motility and epithelial–mesenchymal transition in nude mice [95]. Nanomolar concentrations of rotenone significantly reduced the migration/invasion of A549 and H1650 cells and their cisplatin-resistant counterparts. Because rotenone at tested nanomolar concentrations did not cause cell death, the decreased migration of the treated cells was not a consequence of reduced viability. Although many ETC inhibitors including rotenone are toxic, therapeutic windows may exist for their low-dose use in cancer therapy [96].

Metformin, nontoxic CI inhibitor, was found to suppress *in vivo* invasion and metastasis of esophageal squamous cell carcinoma [97–99], breast carcinoma [100,101], non-small cell lung cancer [102], cervical cancer [103], liver cancer [104], pancreatic cancer [105], and ovarian cancer [106]. It was found that metformin may be used as adjuvant therapy in cancer treatment [107] because it can increase the sensitivity of conventional chemotherapy drugs, making it a successful example of drug repurposing for anticancer and antimetastatic treatment. In addition, several clinical studies have confirmed that it can improve patient prognosis, which may be achieved by inhibiting cancer invasion and migration [108]. However, there is a concern about the resulting concentrations of metformin in tumor tissues and their ability to inhibit CI. This has led to the development of mitochondria-targeted analogs of metformin [109]. Another CI inhibitor, IACS-010759, is currently being evaluated in Phase 1 clinical trials in relapsed/refractory acute myeloid leukemia and solid tumors [110]. Other CI inhibitors such as BAY87-2243, AG311, and kalkitoxin can inhibit cell migration and invasion through deactivation of hypoxia-inducible factor 1α (HIF-1α) signaling that causes hypoxia-induced motility and invasiveness [111]. Inhibition of mitochondrial oxygen consumption by these inhibitors was found to reduce HIF-1α stabilization by increasing oxygen tension under hypoxic conditions [112–114]. Despite the lack of toxicity in mice, an initial Phase 1 trial of BAY87-2243 in human (NCT01297530) needed to be terminated owing to unexpected toxicity and safety issues [115].

Tamoxifen (TAM), an established agent for the treatment of estrogen receptor (ER)-positive breast cancers, was shown to inhibit CI at the flavin site [116]. Some results indicate that TAM can enhance Twist1 degradation and consequently suppress cancer cell invasion and metastasis, suggesting that TAM can be used not only to treat ER-positive breast cancers but also to reduce Twist1-mediated invasion and metastasis in ER-negative breast cancers [117].

The fact that cancer cells exhibit a higher mitochondrial membrane potential ($\Delta\psi_{mt}$) compared to normal cells has allowed selective targeting of cancer cell mitochondria and conjugation of mitochondria-targeting molecules, such as triphenylphosphonium (TPP), mitochondrial-penetrating peptide, rhodamine 123, and SS peptides, with FDA-approved anticancer drugs [118]. TPP-tagged TAM (MitoTam) suppressed not only the primary tumor growth but also the metastatic burden in blood, lung, and liver in the experimental 4T1 model of human epidermal growth factor receptor 2 (HER2)-high metastatic breast carcinoma in mice [119]. MitoTam has been tested in a Phase 1 trial with encouraging outcomes and is entering Phase 2 trials [118].

Promising migrastatic properties are also exhibited by arsenic trioxide (As_2O_3), an FDA-approved complex IV inhibitor used for the treatment of acute promyelocytic leukemia that is being investigated in other cancer types. As_2O_3 reduces the invasive and metastatic properties of cervical cancer cells both *in vitro* and *in vivo* [120], and mitochondrial cardiolipin-binding panthamethinium salts (PMSs) [121], which accumulate in the mitochondria of cancer cells, are reported to diminish their motility, migration, and invasive potential [122]. Furthermore, PMSs cause the retraction of mitochondria from the leading edge of the cell, which can diminish the ability of cancer cells to migrate [86]. Liver metastatic colonization and metastatic potential of colorectal cancer was also strongly impaired by dihydroorotate dehydrogenase (DHODH) inhibition by leflunomide [123]. DHODH converts dihydroorotate to orotate, and the resulting two electrons are transferred to ubiquinone in the respiratory chain. DHODH-driven pyrimidine biosynthesis is probably one of the key pathways linking respiration to cancerogenesis [84]. Leflunomide also inhibited transendothelial migration of peripheral blood mononuclear cells [124].

Some compounds, such as second-generation isoflavone ME-344, can inhibit several mitochondrial complexes simultaneously. ME-344 has a recent history of both preclinical and early clinical testing (NCT01544322) [125]. The drug has unusual cytotoxicity profiles, where cancer cell lines can be either intrinsically sensitive or resistant. In addition to inhibition of respiratory complexes I–V, the mechanisms of action also include inhibition of tubulin polymerization, reduction in ATP production, activation of AMPK leading to induction of autophagy, increased production of mitochondrial reactive oxygen species (ROS), inhibition of heme oxygenase-1, and induction of its translocation to mitochondria [126]. ME-344 showed anticancer activity in HER2-negative breast tumors, particularly after vascular normalization and tissue reoxygenation induced by bevacizumab [125].

Another group of drugs that can interfere with electron flow along the ETC are substances that disrupt mitochondrial metal homeostasis, such as elesclomol (STA-4783; the active form is a deprotonated copper chelate) [127,128] and mitochondria-targeted deferoxamine (mitoDFO) [129]. Such interference leads to the inhibition of ETC activity and oxidative phosphorylation followed by elevated levels of electron leakage and ROS formation [128,129]. In a Phase 3 clinical trial, elesclomol showed a promising effect in the treatment of metastatic melanoma (in combination with paclitaxel). However, this effect was limited to patients with low serum lactate dehydrogenase (LDH) levels [130]. High serum levels of LDH are thought to reflect a type of melanoma with decreased reliance on OXPHOS [131]. Deferoxamine (DFO) represents an FDA-approved iron chelator that is widely used for the treatment of iron overload diseases. Several clinical studies have shown that DFO also exhibits antitumor effects in patients with neuroblastoma and hepatocellular carcinoma [132,133]. MitoDFO represents a way to deprive cancer cells of biologically active iron in mitochondria, which can stop their proliferation, migration, and metastatic processes without disrupting systemic iron metabolism. MitoDFO significantly suppressed tumor growth and metastasis *in vivo* [129]. Another iron chelator, deferasirox (DFX), causes partial uncoupling and dramatic swelling of mitochondria but without membrane depolarization or opening of the mitochondrial permeability transition pore [134]. DFX was shown to suppress the motility of cancer cells by reducing Cdc42 and Rac1 activation in pancreatic cancer cell lines [135]. Furthermore, orally administrated DFX potently inhibited lung carcinoma (DMS-53) xenograft growth in nude mice, with preservation of normal tissue histology in other tissues [136]. Although the iron chelator VLX600 looked promising during *in vitro* experiments [137], the Phase 1 clinical trial of this chelator (NCT02222363) was terminated because of lack of efficacy. The clinical failure may be caused by poor drug accumulation, which can be addressed by mitochondrial targeting [118].

Some substances originally developed for other purposes, such as inhibitors of Hedgehog signaling, also have mitochondria-targeting effects. Cyclopamine tartrate and SANT1 strongly interfere with mitochondrial function and suppress aerobic respiration in lung cancer cells [138]. Inhibition of mitochondrial respiration interferes strongly with the proliferation, colony formation, migration, and invasion in these cells. It also delays the growth and progression of non-small cell lung cancer in subcutaneous as well as orthotopic lung tumor xenografts [139,140].

However, targeting mitochondria has some limitations. Cancer cells are flexible and can compensate for the loss of mitochondrial ATP production by exogenous mitochondrial transfer from host cells in the tumor microenvironment. By this mechanism compromised respiratory function can be reestablished [83,141]. Because mitochondrial transfer via tunneling nanotubes (TnTs) can play a role in this horizontal transfer [142], drugs that inhibit TnT formation such as metformin and everolimus [143] can be synergistic with mitochondria-targeting agents. Inhibition of OXPHOS also induced mitochondrial fission and increased the numbers of functional mitochondria in acute myeloid leukemia cells [141]. Therapeutic drugs targeting ATP availability and mitochondrial metabolism with potential for repurposing towards migrastatic therapy are summarized in Table 2.

Table 2. Therapeutic drugs targeting ATP availability and mitochondrial metabolism with the repurposing potential for migrastatic therapy[a,b]

Pathway	Targeted molecule	Compound	Status	Refs
Mitochondrial respiration	Mitochondrial complex I	Metformin	Clinical trials (Phase 2 and 3; ovarian and breast cancer); NCT01579812, NCT01101438	[108,165]
	Mitochondrial complex I	Rotenone	Animal models	[95]
	Mitochondrial complex I	IACS-010759	Clinical trials (Phase 1; advanced cancers, AML); NCT03291938, NCT02882321	[110]
	Mitochondrial complex I	AG311	Animal models	[113]
	Mitochondrial complex I	Tamoxifen	FDA-approved (breast cancer)	[116]
	Mitochondrial complex I	MitoTam	Clinical trials (Phase 1/1b; metastatic cancers); EudraCT 2017-004441-25	[118,166]
	Mitochondrial complex II subunit SDHB	Gamitrinib	Clinical trials (Phase 1; advanced cancers); NCT04827810	[167]
	Mitochondrial complex IV	Arsenic trioxide	FDA-approved (acute promyelocytic leukemia)	[120]
	DHODH	Leflunomide	FDA-approved (rheumatoid arthritis)	[123]
	Mitochondrial complexes	ME-344	Clinical trials (Phase 1; solid cancers); NCT01544322	[125]
	Hedgehog signaling	Cyclopamine tartrate	Animal models	[139,140]
Mitochondrial motility	Miro1	Miro1 reducer	Animal models	[168]
Mitochondrial metal homeostasis	Copper	Elesclomol	Clinical trials (Phase 2 and 3; ovarian cancer, melanoma); NCT00888615, NCT00522834	[130]
	Iron	Deferoxamine	FDA-approved (iron overload)	[132,133]
	Iron	MitoDFO	Animal models	[129]
	Iron	Deferasirox	Clinical trials (Phase 3; iron overload); NCT00171821	[136]

[a]Abbreviations: AML, acute myeloid leukemia; DHODH, dihydroorotate dehydrogenase; EudraCT, EU Drug Regulating Authorities Clinical Trials Database; MitoDFO, mitochondria-targeted deferoxamine; MitoTam, mitochondria-targeted tamoxifen.
[b]Status: 'clinical trials' means that the drug was involved in any type of clinical trial (not necessarily a clinical test for cancer). We only considered agents that have been tested in animals or in clinical trials because their future therapeutic potential is higher and their repurposing is easier. The references cited refer to the use of these drugs in cancer therapy. The term 'solid cancer' refers to a solid mass of cancer cells that grows in vital organs, whereas 'liquid cancers' occur in the blood, bone marrow, or lymph nodes.

Concluding remarks and future perspectives

Although systemic metastases are responsible for ~90% of cancer deaths, most clinical trials in cancer currently do not involve direct targeting of the metastatic process. Studies of tumor cell kinetics indicate that even small tumors can have the capacity to generate metastases. Consequently, the tumor shrinkage observed in preclinical studies may not be an adequate marker of the future clinical benefit of the tested anticancer drugs, and greater emphasis should be placed on the migrastatic and anti-invasive potential of the studied therapeutic drugs.

Given the preventive action of migrastatics, the effectiveness of migrastatic strategies cannot easily be evaluated in clinical trials with cancer patients who already have an advanced form of the disease. Furthermore, tumor size, cancer cell proliferation, and cell death markers are unlikely to be influenced by migrastatic therapy, which can lead to the false assumption that a given therapy is ineffective. This premature elimination of drugs from further research should be avoided in the future, and selection of proper clinical endpoints for antimetastatic treatment such as metastasis-free survival should be adopted.

Despite these obstacles, we believe that migrastatic therapy holds great promise in the future. The big advantage of migrastatic therapy is that selection pressures caused by this type of therapy may not cause resistance to conventional therapies based on antiproliferative effects because the targeted mechanisms are of a completely different nature [144]. Migrastatic therapy can also support active surveillance approaches, restrict infiltration of adjacent tissues and local invasion, and block further dissemination of cancer cells in patients with advanced cancer. Limiting cancer cell motility may also reduce evolution towards progressively metastatic phenotypes [2].

In conclusion, migrastatic strategies represent a unique approach that could prevent the development of systemic cancer disease and limit cancer-related death. A successful migrastatic therapy administered simultaneously with standard therapy could show a synergistic effect and therefore reduce the need for aggressive high-dose cytotoxic therapies that are currently used to combat metastatic dissemination. The best targets for migrastatic therapy seem to be those that are required by all forms of motility, such as ATP availability and mitochondrial metabolism, as well as cytoskeletal dynamics and cell contractility. ATP-depletion strategies can be complemented by approaches that force cells to use more energy-consuming ways of moving or that prevent them from moving in less energy-consuming ways. The fields of molecular biology and oncology should tightly cooperate to explore the evolution of tumors and the exact mechanisms underlying metastatic dissemination. This type of research could provide innovative and exciting new ways to think about therapeutic combinations for treating metastatic disease (see Outstanding questions).

Outstanding questions

Studies on tumor cell kinetics indicate that even small tumors can have the capacity to generate metastases. Consequently, is tumor shrinkage observed in preclinical studies an adequate marker of the future clinical benefit of the tested anticancer drugs?

Because the molecular mechanisms that enable cellular proliferation differ from those that enable cell migration or invasion, should we not evaluate candidate anticancer drugs for their ability to stop the invasion or migration of cancer cells even if they are not cytotoxic to cancer cells and do not have a high antiproliferative effect?

Can migrastatic strategies that complement conventional therapy decrease the risk of adverse effects of anticancer treatment?

Several studies have shown that cancerogenesis is accompanied by changes in the content and composition of cardiolipins (mitochondrial phospholipids that constitute ~20% of the inner mitochondria membrane). Can mitochondrial targeting of therapeutic agents be improved by using cancer-specific cardiolipin-binding molecules?

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Declaration of interests

The authors declare no competing interests.

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