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The pan-therapeutic resistance of disseminated tumor cells: role of phenotypic plasticity and the metastatic microenvironment

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Abstract

Cancer metastasis is the leading cause of mortality in patients with solid tumors. The majority of these deaths are associated with metastatic disease that occurs after a period of clinical remission, anywhere from months to decades following removal of the primary mass. This dormancy is prominent in cancers of the breast and prostate among others, leaving the survivors uncertain about their longer-term prognosis. The most daunting aspect of this dormancy and re-emergence is that the micrometastases in particular, and even large lethal outgrowths are often show resistance to agents to which they have not been exposed. This suggests that in addition to specific mutations that target single agents, there also exist adaptive mechanisms that provide this pan-resistance. Potential molecular underpinnings of which are the topic of this review.

1. Introduction

Metastatic cancers remain largely incurable with most therapies being temporizing and/or palliative. The therapeutic treatment used are by in large the same as those for primary tumor. Even in the face of objective response, and when the primary tumor does shrink in response to therapies, the overwhelming majority recur and show broader resistance against therapies [1–3]. With respect to chemotherapy, metastatic lesions are frequently resistant [4–6] and more troublingly is that this treatment can facilitate metastatic tumor growth and drug resistance. Chemotherapy elicits the secretion of various paracrine factors from the stromal cells in the surrounding microenvironment which promote resistance [7–10]. For targeted

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therapy, a few patients achieve a complete response and others a partial response or stable disease, but the majority continue to progress [11, 12]. Holding some promise, immunotherapies have come to the forefront due to unprecedented durable response rates for select carcinoma types [13–15]. However, the majority of patients do not benefit and of those that do respond (being only one quarter or fewer of treated patients), a substantial portion relapse after a period months to years [16, 17].

Mechanisms underlying resistance to chemotherapies, targeted therapies and immunotherapies in metastatic tumors are often assumed to occur via Darwinian selection of mutants. However, genomic studies have revealed that primary tumors and metastases are closely related from a genetic standpoint [18] and failed to identify unique mutations associated with metastatic propensity [19–21]. This would suggest that disseminated tumor cells (DTCs) do not necessarily acquire unique cell-intrinsic effector functions but instead draw from other resources preexisting in normal cells for pathophysiological function at various stages of development. That is to say, all the cell-intrinsic resources required for dissemination are available in the physiological setting, and tumor cells likely only adjust their expression level and combination to hijack them to their advantage.

Within this review, we will discuss the concept of pan-resistance of DTCs, micrometastases and macrometastases. We discuss that the pan-resistance described above is not merely a unicellular resistance mechanism brought about by clonal expansion of mutant cells and focus on how therapeutic resistance is significantly influenced by the powerful, bidirectional relationship that exists between DTCs and the metastatic microenvironment (MME) [22]. We also propose that pan-resistance pertains to multiple aspects; not merely resistance to multiple drugs but to multiple therapeutic types too.

2. Metastasis associated cancer cell plasticity

2.1 Metastatic cascade

Metastasis is the result of DTCs that initiate new lesions in distant organ sites. Briefly, the cascade involves escape from the primary tumor, intravasation into the circulation, systemic dissemination followed by extravasation and colonization of a distant organ. DTCs may then outgrow immediately into an overt metastasis or establish as a dormant, non-proliferating cell or micrometastatic nodule [23, 24] (Fig 1).

Metastasis can be an early event such that even small tumors < 5mm can establish metastasis long before they become detectable at the primary site [25, 26]. This appears to be the case for some types of solid cancers (*e.g.* breast, renal cell, lung) [27–29] whereas evidence for others indicates a late escape (*e.g.* prostate, colorectal and pancreatic) [30–32]. Regardless, it is clear that undetected DTCs are the cause of latent metastatic disease. Throughout the course of the metastatic cascade, the microenvironment around the tumor cells is continually changing and evolving. Subsequently, it is exceedingly important to take into account the dynamic influence it has on protecting metastatic cells from the immune system and therapies whether that be chemo-, target and immunotherapy. The non-genetic factors as well as the stochastic phenotypic switching in DTCs post-extravasation, which results from this relationship, will be a focus of this review.

2.2 Epithelial/mesenchymal cell plasticity

Successful metastatic seeding of distant organs depends on the capability of cancer cells escaping from the primary tumor, surviving during migration and colonization in the ectopic sites. Cell epithelial/mesenchymal plasticity, particularly in carcinomas, confers the ability for cells to change behaviors in terms of movement through tissues and avoidance of contact inhibition of proliferation. The concept of epithelial-mesenchymal transition (EMT) evolved from initial observations that embryonic and adult epithelial cells converted to migratory and invasive fibroblast-like cells when embedded in 3D collagen gels [33]. Since then, EMT has been well studied, given way from “transformation” to “transition”, and “plasticity” more recently, in development, wound healing, fibrosis, and cancer metastasis [34]. Different from EMT in embryogenesis and organ development, which is subtle and controlled and reflects a defined set of transcriptomic and proteomic dichotomies, cancer-associated EMT (cEMT) is aggressive and uncontrolled [35]. Instead of a complete transition to a mesenchymal state as noted during development, carcinoma cells often exhibit a spectrum of epithelial/mesenchymal phenotype. Compared to extremely Epithelial (E) or mesenchymal (M), the hybrid E/M state has been shown to be stable over multiple passages *in vitro* and “metastable”. Such hybrid E/M state cancer cells are broadly existing in primary and metastases, as well as circulating tumor cells [36–42]. It is even questioned if there are any fully E or M phenotypes in cancer. Therefore, cEMT, and its reverse cancer-associated mesenchymal-epithelial transition (cMET) reflect the relative state shift with respect to the original tumor cells.

In development, the M versus E phenotypic state is characterized by the expression of a set of transcription factors including Twist, Snail1/2 (Slug) and Zeb1, along with the intermediate filament protein vimentin replacing cytokeratins, and the loss of E-cadherin [43–45]. However, in cancer-associated switching, the fidelity of co-expression is lost, rendering definitive phenotypic assignment inconclusive [44, 45]. One consistent marker of the E versus M state, is the cell-cell cohesion homotypic binding receptor E-cadherin [24]. As this is the first receptor to bring cell sheets together and enable the formation of adhesion and then tight junctions that allow for cell polarization, the sine qua non of an “Epithelial” phenotype, its presence or loss from the cell surface can be used to define E and M, respectively [24]. In fact, forced loss of E-cadherin drives a cancer-associated EMT and metastatic behaviors [46], while forced re-expression of this receptor can be viewed as a metastasis suppressor [47–50]. For this discussion, and in recognition of the fluid nature of cancer cell phenotypes, E will be defined as cell surface ligandated E-cadherin, and M by its absence.

3. Early stage metastatic lesions

By this step of colonization, DTCs have escaped from the primary mass, survived the sheer forces and thrombotic events and hematopoietic cells while in the circulation and are now confronted with a foreign and hostile environment for seeding [23, 24, 51]. Within this section, we discuss the how DTCs survive through communications with the MME to successfully colonize at single cells or form micrometastases as well as the molecular and cellular mechanisms that confer pan-resistance at this stage of the cascade.

Presently, it is not possible to determine the presence of dormant DTCs in patients but evidence to indicate that dormant DTCs are resistant to common therapies derives from multiple observations where they have been recovered from bone marrow aspirates of patients several years after systemic therapeutic regimes (both chemotherapy and targeted therapies) [52–55]. While one aspect may be that the dormant cells are often out of the cell cycle, and most chemotherapies and biological agents target dividing cells [56, 57], this is not the complete story, as even growing micrometastases show similar chemoresistance [58]. We also discuss that dormancy involves the convergence of multiple mechanisms; cellular proliferation, angiogenesis and immunological factors, all of which have made the task of finding possibilities for therapeutic targeting and efficacy exceptionally difficult.

3.1 E-cadherin as a mediator of dormancy and chemoresistance

Though under provocative discussions on whether any of these phenotypic transitions are indispensable for metastases formation [34, 44, 45], it is well accepted cEMT enhances the tumor cells capability of migration to escape from the primary sites. It is also becoming acknowledged that a cMET aids survival and colonization in the metastatic sites [23, 24] (Fig 2).

After an initial cEMT involved in tumor cell breaking away from the primary mass and intravasating into a vascular conduit, the DTC is confronted with a foreign and hostile environment for seeding. Only a diminishing fraction (on the order of 0.01% or less; based on *in vivo* mouse models [59]) can accomplish seeding and survival. Ectopic seeding requires a cMET with re-expression of E-cadherin. This is based upon the observation that many small metastases express epithelial markers [60, 61]. Chao *et al.*, examined E-cadherin expression in primary tumors and matched metastases and found that 62% of cases had increased E-cadherin at the metastatic sites compared to the primary tumors [62]. More importantly, not only was E-cadherin presented on the cell membrane in the metastases, whereas it was mainly cytoplasmic in the primary tumors; nearly all of the smallest metastases were epithelial in this manner, with the fraction of presentation decreasing as the nodule became larger, indicating a secondary cEMT being involved in the outgrowth. The cMET is also a partial transition, in that these tumor nodules simultaneously express mesenchymal markers [62]. This is also true in experimental prostate cancer, with E-cadherin expression level is conversely associated with tumor size [58, 62].

This phenotypic switching begs the question of the inductive signals that drive the changes, as E-cadherin is downregulated at multiple levels of control, but is neither mutated nor deleted genetically [24]. In the primary site, cancer-associated fibroblasts [63, 64] and macrophages [65, 66] are considered to express signals that drive cell scattering. Much less is known about the microenvironment at the site of initial intravasation and seeding that allows for cMET and cell survival.

The liver and lung are epithelial organs, in which parenchyma express E-cadherin and can form cell-heterotypic E-cadherin homotypic binding. Tumors cells regain E-cadherin at the cell surface membrane in the lung in patients and *in vivo*, and increase E-cadherin, be more cuboidal morphology when co-culturing with normal lung epithelial cells *in vitro*, similar to the situation in liver [47, 67]. Surprisingly, this cMET even occurs in the brain and bone

marrow, containing mesenchymal environments that do not present E-cadherin on their resident cells for homotypic ligation and signaling [61, 62].

In prostate cancer, we have shown that E-cadherin can be presented on the membrane in the ectopic site upon abrogation of the EGF receptor (EGFR) activating autocrine loop that initially provides for a mesenchymal phenotype [68]. The blockade of EGFR by hepatocytes, allows for catenin stabilization of E-cadherin on the cell surface, and cell-homotypic and -heterotypic binding with functional gap junctions being formed with the hepatocytes [69]. In breast cancer cells, E-cadherin is downregulated by promoter hypermethylation [47], in addition to EGFR autocrine signaling. Still, the liver microenvironment can revert this suppression, but with breast cancer, this requires limited carcinoma cell proliferation to lose the imprinted methylation.

In both breast and prostate cancers other transcriptional regulators also play a role in further suppressing E-cadherin. Kaiso binds to hypermethylated DNA and prevents transcription, leading to a mesenchymal phenotype [70–72]. However, as Kaiso nuclear localization occurs downstream of E-cadherin abrogation, re-establishing E-cadherin signaling then eliminates this blockade of transcription.

This re-expression of E-cadherin during the earliest stages of metastatic seeding may provide for pan-resistance in two ways. The simplest is that as the carcinoma cells undergo an epithelial reversion, the fraction and velocity of cells actively proliferating is reduced. As most chemotherapeutics and biologics (such as CDK inhibitors and anti-RPTK cascades) target cells in cycle [73–75] this would limit the effectiveness of all these therapies. Even agents that cause cell damage or stress (*e.g.* metabolic blockade and proteasome inhibitors) would be rendered less efficacious as the quiescent cell has lower metabolic requirements and extended repair times.

However, this can only be a part of the story, as we have found that E-cadherin expression, and the accompanying cMET, provides for relative protection from multiple chemotherapies independent of proliferation [58, 62]. In experimental models of both breast and prostate cancers, the E micrometastases were protected from induced death while the M phenotype metastases (in both liver and lung) and the primary tumors were effectively treated. This was recapitulated in cell culture and mixed co-culture systems wherein proliferating E-cadherin positive carcinoma cells were not induced to apoptosis whereas the neighboring mesenchymal cells were despite similar proliferation fractions.

This pan resistance results from E-cadherin signaling through canonical survival pathways including PI3-kinase/AKT and MEK/Erk [58, 62]. Superficially, this may create a conundrum as these same pathways along with the p38 Erk pathway, prevent cMET, and their downregulation in the micrometastatic microenvironment is what enables the shift to E with re-expression of E-cadherin [76]. But it appears that the signaling through ligandated E-cadherin is qualitatively and functionally distinct in that it is a low level, barely detectable in non-stressed cells but evident when the cells are challenged by chemotherapies and stressors [58]. The low level and tonicity of the activation of the intermediary kinases is similar to that noted with EGFR signaling when restricted to the cell surface by either tethered ligand or

the matricellular proteins tenascin C and laminin V; this signaling enhances survival of stem cells challenged by death signals both in vivo and in vitro, while the pulsatile high level activation by growth factors actually sensitize the cells to death [77–80].

This mode of protection opens new pathways to reverting the chemoresistance of dormancy in that targeting these key kinases would re-sensitize tumors to normal chemotherapy. As MEK inhibitors are in clinical use, particularly for metastatic melanoma, and various inhibitors of the PI3-kinase/AKT pathway have been developed for human use (though limited by systemic toxicity when used at high doses), the use of low doses of these agents would serve to ‘rescue’ classical therapies for increased efficacy against the dormant micrometastases [58].

3.2 Dormancy signals from the MME

Following dissemination, the vascular basement membrane is the first thing DTCs encounter. Consequently, it is not surprising that the common metastatic locations for solid tumors are those with a mostly vascular primary basement membrane (*e.g.* lung, liver, brain and bone marrow) [81]. This perivascular niche has been shown to confer growth arrest and survival in DTCs [82, 83]. In addition to E-cadherin, various tissue-specific dormancy-inducing signals have also been associated with promoting dormancy (*e.g.* BMP7 [84], BMP4 [85], TSP-1 [82]) CXCR12/CXCR4 [86]). Mechanistically, many of the dormancy-inducing signals lead to activation of the p38 MAPK pathway and in the absence of mitogenic signals; this promotes an ERK^{lo}/p38^{hi} state in DTCs that in turn drives G0/G1 cell cycle arrest and dormancy [87].

Entry into dormancy is also mediated in some instances by interactions with the extracellular matrix (ECM) proteins. Integrin binding to ECM and stromal cells can also cause cell cycle arrest and confer reduced sensitivity to cytotoxic therapies [88]. Notably, although DTCs may up-regulate and alter the repertoire of integrins they express, the ones used to interact with ECM proteins in the MME are typically not mutated and their function remains the same as that for normal cells. Additionally, a collagen I-rich environment suppresses tumor progression via DDR signaling [89, 90]. This provides further credence to the need for a high degree of cellular plasticity exhibited by a DTC rather than genetic mutations being key to surviving the metastatic cascade.

Additional examples have been identified that support the model above wherein active signaling from MME imparts resistance to dormant cells, either in addition to, or predominantly over mitogenic suppression. Resident liver endothelial cells (liver sinusoidal endothelial cells; LSECs) were found to promote survival by inhibiting antitumor immune responses in the early metastatic niche. Interaction of tumor activated-LSECs with lymphocytes decreased their antitumor cytotoxicity and interferon- γ (IFN- γ) secretion [91]. This inhibition of cytotoxic T cells may also further enhance the resistance of dormant cells to immunotherapies, but remains to be determined. DTCs that grow in the bone marrow microenvironment stimulate the host MME to produce survival factors, such as IL-6 and insulin-like growth factors, which protect the tumor cells from cytotoxic drugs [92]. Other recent work has posited that chemotherapy resistant cells predominantly resided in the perivascular niche and the protection from therapy was from the vascular endothelium via

VCAM1 and von Willebrand factor binding. These dormant cells could be sensitized to chemotherapy and bone metastases prevented by inhibiting integrin mediated interactions with VCAM1 and von Willebrand factor [93]. These studies combined with our work with E-cadherin, are starting to shed light on the significant role the MME play in shaping resistance in dormant tumor populations.

3.3 Immune escape mechanisms in dormancy

Little is known about whether and how immune cells interact with dormant DTCs. Reports indicate that dormant DTCs may exhibit immune evasive characteristics as we note above [23] rather than directly immune suppressive characteristics that have been posited to exist in later macrometastases [94]. Emerging evidence showed DTCs reduced or lacked tumor antigen presentation via down-regulation of MHC I, making them unrecognizable by the adaptive immune system [95]. Additionally, breast and lung DTCs were reported to broadly down-regulate NK cell-activating ligands, which are important for recognition and eradication by NK cells. The ability appears to be firmly linked with entrance into dormancy [96]. This is also reflected by reduced PD-L1 on E phenotype metastatic tumor cells [23], and while this may appear to prime cells for immune clearance, the concomitant reduction in MHC I renders these cells invisible to the cytotoxic immune cells, and subvert the efficacy of checkpoint inhibitors currently being applied. This suggests that dormant cells may also be inherently resistant to immune checkpoint inhibitors or vaccination therapies that enhance T cell and NK cell mediated killing.

3.4 Immunosuppressive MME protections in dormancy

Immunosuppressive immune cells are involved from the point of DTCs extravagating into the metastatic organ. They begin by creating a permissible pre-metastatic niche that includes metastasis-associated macrophages (MAMs; mainly M2-like), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) [97, 98]. They are recruited by soluble factors and extracellular vesicles derived from the primary tumor to help form pre-metastatic niches [94]. All three create a protective microenvironment for the DTCs to arrive in to by suppressing the cytotoxic functions of T and/or NK cells and in doing so [99–101]. Those from the myeloid lineage (MAMs and MDSCs) appear important for promoting the colonization and survival of DTCs [97, 102]. MAMs expressing integrin- $\alpha 4$ aid this process by binding VCAM-1 on DTCs which in turn activates survival signaling [103]. Collectively, these cells serve to protect DTCs from a hostile microenvironment as well as surveillance by immune attack, which can also block the effects of immunotherapy and enable colonization and establishment of metastasis [98, 104, 105].

4. Late stage metastatic lesions

Although mechanisms involved in the early stages metastatic progression are partly understood, those that enable or provoke re-emergence of the dormant tumors remain unclear. Studies point towards the microenvironment as being the one with the license to drive the transition, suggesting the progression to an overt metastatic lesion in dormant cells is irrespective of their underlying genetics. From a progression perspective, we know that reactivation and exit from dormancy in DTCs or micrometastases requires a second cEMT

induction, initiation of proliferation, continued survival signaling, and potentially continued evasion of the immune system. The instigators that initiate this sequence of events are beginning to unfold. We and others have shown that it involves disruption of the systemic (macro-environmental level) and local homeostasis (micro-environmental level) [23, 106–109].

Resistance in this stage of the metastatic cascade is conferred via the multicellular nature of the MME (*e.g.* cell-cell and cell-ECM interactions as well as soluble factors and EVs-mediated signaling) and is discussed below (Fig 3). Cellular and epigenetic resistance mechanisms are also barriers but have been discussed extensively elsewhere [4, 6, 11, 13–15, 110].

4.1 Mesenchymal transitions in the MME contain elements of protection from death

A cMErT is required for DTCs landing and seeding in the ectopic organ, whereas a secondary cEMT has been found concomitant with metastatic outgrowth in both mouse model and patients. This is based on the observations that E-cadherin expression levels reversely correlate with tumor size [58, 62]. These growing mesenchymal cells are active in cycle and metabolism, which should be responsive to therapies as the primary lesion. However, this is not the case, and so does even the immediate lethal outgrowth (such as triple negative breast cancer). Rather than exposure-related selection or mutation, signals awakening dormant cells or proliferation promoters from the MME are likely the key player.

Simultaneously with the re-active MME secreting signals that drive the secondary cEMT, these cells also produce matrix elements and signals to provide for survival [23]. Chief among these is the ECM matricellular protein tenascin C, that tonically signals via the EGFR in a cell survival mode [111]. This is in addition to soluble signals, which are noted below in “*MME and initiation of proliferation programs*”.

4.2 Angiogenic switch

In select situations, the exit from dormancy into a proliferative nodule may occur through vascular sprouting and triggering the angiogenic-switch [57, 82, 83]. The relative importance of this event is open to question, as many metastatic tumors coopt existing vasculature, and thus do not require angiogenic expansion [112]. This may explain why angiogenic inhibition has had limited success in the treatment of most metastatic disease, though it is clear that some tumor types are more susceptible to such therapies [113, 114].

Despite the question as to universality of this mechanism, there is evidence that such sprouting may drive emergence [82]. In such a situation, the endothelial cells would not only promote growth by supplying oxygen and nutrients, they also secrete angiocrine factors, which comprise an array of growth factors, trophogens, chemokines, adhesions molecules [115]. In particular, sprouting endothelial cells break dormancy and promote proliferation via secretion of TGF- β 1 and periostin [82]. At this stage of metastasis, extracellular vesicles derived from endothelial cells are thought to confer an increased resistance to multiple chemotherapies [116]. Furthermore, as the nodule transitions from a macrometastasis to an overt metastasis the vascular becomes increasing disorganized and abnormal leading to high

interstitial fluid pressure. Together these two aspects confer a physiological pharmacokinetic resistance mechanism as they limit the ability of drugs to penetrate.

4.3 MME and initiation of proliferation programs

Changes in the MME that disrupt the stable dormant state are likely the main instigators of emergence as well as resistance. It is becoming increasingly evident that the homeostasis of the body as a whole and not simply the local MME is involved in regulating dormancy. In particular the inflammation appears to be a key initiator of emergent growth. We, and others, have shown that a systemic and chronic inflammatory response is linked to re-emergence from dormancy [106–109, 117, 118]. Examples of homeostatic disruption leading to metastatic progression include surgery [118], gut perturbations [106], alcohol [119] and obesity [120].

Recent studies by others and ourselves have identified the resident non-parenchymal cells of a metastatic organ, particularly endothelial cells and stromal cells, as being activated by the disrupted homeostasis and in turn promoting metastasis [82, 106, 117, 121–123]. These studies demonstrate that stressed endothelial cells [123] or stromal elements (*e.g.* hepatic stellate cells and CAFs) [117, 124] in the metastatic host organ respond by producing growth factors (such as EGF receptor ligands) and cytokines and chemokines (*e.g.* IL-8, MCP-1, IL-6) can awaken the dormant cells. Such a re-entry into the cell cycle would be expected to re-sensitize the metastatic cells if the main mode of chemo-resistance was quiescence, but as this is not the case, concomitant changes in MME also need to provide for tumor cell resistance/survival.

In addition to soluble signals, activated stromal cells, particularly cancer associated fibroblasts (CAFs) and hepatic stellate cells, have been shown to secrete high levels of ECM proteins, which create a dense fibrotic MME. They promote outgrowth by producing ECM rich in collagen, fibronectin and laminin in and around the metastasis [125, 126]. Subsequent binding to these ECM components confers resistance to cytotoxic drugs by inhibiting drug-induced apoptosis [127] and increases resistance to targeted antibody therapies [128]. Resistance to therapies also occurs due to the increasing ECM stiffness, leading to pronounced desmoplasia that creates high interstitial pressure and limits drug penetration [8].

4.4 Immunosuppressive MME in late metastases

The immunotherapy resistance noted in late stage metastasis is posited to occur due to the creation of an overall suppressive MME. Evidence is emerging that points towards the innate immune system as being the main immune protagonist in late stage MME [129, 130]. Research has predominantly focused the activity of macrophages at the primary site, but data is beginning to emerge on the specific role of macrophages in the MME (recently extensively reviewed [131]). Consistently, innate immune cells are widely observed to varying degrees almost all metastatic tumors and can represent up to half of the cells in a tumor nodule [132], while the presence of T cells is variable [133–135]. In some tumors there is a robust infiltration of T cells while in others they are almost absent or spatially restricted to the tumor margin [133–135], that would limit functionality in clearing the tumor.

With respect to emergence from dormancy, the systemic inflammation described above has also been shown to recruit macrophages, which subsequently trigger the outgrowth of previously dormant tumor cells [118]. Our investigations indicate this is likely induced by the M2-like macrophages and is imparted via induction of a second cEMT [122]. Others found MAMs could be recruited to the MME by DTC-derived endothelin-1 in the lung wherein they secreted trophic and immunosuppressive factors (*e.g.* MCP-1 and IL-6) that were essential for the transition of micrometastases to overt metastases [136]. Resident macrophages in the liver also directly stimulate proliferation through secretion of HGF, TNF- α , IL1 β and IL-10 [137, 138]. Notably, the secretion of IL-6 by macrophages also confers chemoresistance [139]. MAMs also indirectly promote sustained metastatic outgrowth by activating stromal cells that then secrete ECM proteins (*e.g.* periostin), resulting in a fibrotic MME [140] and through inhibition of tumoricidal immune response [141]. Although the role of MAMs in promoting and sustaining metastatic growth is gaining ground, their role in mediating therapeutic resistance at metastatic sites remains unclear, and represents an opportunity for further study and potential therapeutic approach.

The MDSC innate immune cells also may promote both outgrowth and therapeutic resistance. Evidence suggests they are main orchestrators of immune suppression [142, 143]. Their presence in metastatic lesions correlates with advanced disease stage [144], poor responses to chemotherapy [145, 146] and resistance to immune checkpoint inhibitors [147, 148]. Furthermore, in a murine model, elimination of MDSCs led to cures of experimental metastatic tumors [148]. Induction of metastatic outgrowth was reported to occur via mutual activation loop with metastatic cells involving IL-6 [149]. MDSCs are able to generate and enhance suppressive microenvironment via various mechanisms, including expression of PD-L1 [150], inducible nitric oxide synthase (iNOS) [151], arginase-1 [152], indoleamine 2,3-dioxygenase (IDO) [153] and secretion osteopontin [101]. These mechanisms serve to strongly inhibit anti-tumor activities of T and NK cells and stimulate Treg cells; this creates significant obstacles for immune checkpoint blockade therapies.

5.0 Conclusions/perspectives/future

The microenvironment that is part and parcel of all tumor organoids during progression provides not just the support for the disseminated carcinoma cells, but also the protection of these cells from dying in these foreign hostile milieus. Unfortunately, the same strategies that confer survival on a vanishingly small fraction of successful metastatic cells also lead to generalized resistance to therapies. That the disseminated cells must overcome a nutritionally and metabolically challenging microenvironment is linked to chemo- and targeted-therapy resistance, with the avoidance of the intrinsic inflammatory response being phenocopied as immune-therapy escape. The molecular bases of these survival strategies are both multifaceted and changing from the earliest stages of seeding, through dormancy, and persisting in aggressive outgrowth.

The plastic and pleiotropic nature of survival in the face of innate and induced/pharmaceutical death signals presents challenges to effective therapies. This is particularly problematic as most persons harbor multiple metastases (including in diverse organs) that often reside at different stages of dormancy and outgrowth. Thus, targeting one organ or

progression stage is likely to be temporizing at best, as the ‘non-targeted’ nodules will persist. Further confounding decisions as to whether and how to treat is the fact that these therapies are not just damaging to non-tumor tissues, but may aggravate those nodules not cleared. This is noted as hyperproliferative disease in the face of immune checkpoint blockade therapy [154], or adaptive cEMT as a consequence of chemotherapy [155], or both [156].

This complexity of therapeutic escape being an integral feature (and not a ‘bug’) of the cancer progression cascade calls for a number fundamental principles in developing curative or persistent treatment regimens. Clinical trials are needed to determine if treatments should target systemic organ changes [106], or try to maintain non-emergent nodule in their dormant state [157]. It is only with a better understanding of the underlying tumor cell and organ biology that we can develop the new diagnostic, theragnostic and interventional approaches that may turn metastatic cancer into a manageable chronic condition rather than a lethal progression.

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Fig.1.

Metastatic cascade post-extravasation. After extravasation, the MME modulates tumor cell plasticity, dormancy and emergence. Successful colonization by DTCs and entrance into a dormant state involves a cancer-associated mesenchymal to epithelial reverting transition (cMErT) and establishing heterotypic E-cadherin connections with the resident cells of the metastatic organ. After a period of time, dormant cells may be stimulated by inflammatory signals to outgrow and undergo a second cancer-associated epithelial to mesenchymal transition (cEMT). The inflammatory signals are produced following homeostatic disruption (either systemically or locally). They may act to stimulate outgrowth of dormant cells directly or indirectly by activating innate immune or stromal cells in the MME which then produce mitogenic signals. Made with Affinity Designer 1.7.0.

Fig. 2.

E-cadherin mediates micrometastases dormancy and chemoresistance. (A) An overview schematic depicting entry into dormancy and acquisition of chemoresistance. (B) E-cadherin promotes cell survival independent of cell arrest or proliferation. E-cadherin, green; cleaved caspase-3, red; EdU, white; DAPI, blue. White arrow shows proliferating cells; yellow arrow shows nonproliferating cells. (C) E-cadherin protects tumor cells in the MME from chemotherapy. Representative images of DU145 prostate cancer cells and cleaved caspase-3 on sister sections of the liver. Same tumor on the sister sections is lined by same color. (B&C) Adapted from [58]. Made with Affinity Designer 1.7.0.

Fig.3.

Mechanisms of resistance conferred by the MME. (A) Chemotherapeutic resistance observed in dormant cells may be conferred by the MME through E-cadherin re-expression, interactions with endothelial cells (ECs), tumor cells integrin binding to extracellular matrix (ECM), and signals, such as IL-6 and IGFs from bone marrow, contribute chemoresistance. Immune recognition and efficacy of immunotherapy is limited through downregulation of immune target molecules (e.g. PD-L1 and MHC I) and possibly some immunosuppressive effects of ECs, macrophages, myeloid derived suppressor cells (MDSCs) and regulatory T cells (Tregs) which suppress the cytotoxic activity or induce apoptosis of anti-tumor T cells and NK cells. (B) Chemotherapeutic resistance observed in outgrowing metastases may be conferred through creation of dense ECM with survival signals from matricellular molecules, tenascin C in particular, and factors produced by activated stromal cells or innate immune cells. Despite upregulated expression of MHC I and PD-L1 on the tumor cells, efficacy of immunotherapy is inhibited through immunosuppressive effects of macrophages, MDSCs and regulatory T cells. Macrophages produced numerous immunosuppressive molecules, while MDSCs produce inflammatory mediators which lead to apoptosis of T and NK cells. MDSCs and Tregs express immune checkpoint proteins PD-L1 and CTLA-4, respectively, which suppress effector T cells. Insulin like growth factors (IGFs); Von

Willebrand factor (vWf); Indoleamine 2,3-dioxygenase (IDO); cancer –associated fibroblasts (CAFs). Made with Affinity Designer 1.7.0.

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