

Bio-mechanistic Insight on Cancer Cells Metastatic Inefficiency

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Abstract: - Cancer metastasis has over the years remain the major causes of cancer death. The biochemical cascade of cancer metastasis flows through a sequence of events involving loss of cellular adhesion, increased motility and invasiveness, entry and survival in the circulation, exit into new tissue (extravasation), and eventual colonization of a distant site. Evasion of the inherent mechanisms halting transformed cells from primary site disseminating to colonize at the secondary or distant site is the hallmark of metastasis. However, metastasis is not an efficient process as most transformed cells (cancer cells) leaving the primary site do not metastasize to distant organs. The all-out inefficiency in metastatic processes are due to myriads of inherent biophysiological homeostatic mechanisms recruited against metastatic cells which involves the switch-on mechanisms of metastatic suppressor genes (MSGs), programmed cell death, immunosurveillance system, angiogenic latency and tumour growth arrest, interstitial pressure, haemodynamic forces and shearing. Inability of all the metastasizing cells to escape, evade or co-opt multiple barriers refined by the inherent programmed homeostatic mechanisms delineate the central paradigm that cancer metastases is an inefficient process. The few transformed cells that successfully metastasize to distant organs leads to cancer death. The “chameleon nature” metastatic cells (evasion of apoptosis, sustained angiogenesis, self-sufficiency in growth signal, tissue invasion and metastasis, limitless potentials for replication, insensitive to antigrowth signal) remain one of the major challenges in the treatment of cancer patient as the development of a potent anti-cancer therapy specific for cancer cells without affecting normal cells (healthy bystander cells) still remains a “holy grail”. However, a pellucid insight on the biomechanistic events delineating metastatic inefficiency will offer a new insight in developing a more potent therapy specific for malignant cells. This worked reviewed the biomechanistic events involved in metastatic inefficiency that may pave the way for researchers to uncover the critical areas in the development of a potent anti-cancer therapy.

Keywords: Metastatic suppressor gene, Tumorigenicity, Metastatic inefficiency, Apoptosis, Malignant cell, Tumour dormancy, Immunosurveillance.

I. INTRODUCTION

Metastasis is a crucial hallmark of cancer progression which involves numerous factors including the degradation of the extracellular matrix (ECM), the epithelial-to-mesenchymal transition (EMT), tumor angiogenesis, development of an inflammatory tumor microenvironment,

and defects in programmed cell death. Approximately 90% of all cancer-related death is due to metastases [1]. Metastatic processes in cancer follows a sequential step called “metastatic cascade”. Its occurrence is a necessary break of the normal homeostatic mechanisms, leading to a rearrangement of the stromal tissue adjacent to the primary tumor. It is now well established that for this process, cancer cells need to acquire additional properties, which confer the capacity to invade the extracellular matrix, migrate, invade blood and lymph vessels, adhere, survive in target organs, grow and promote “organogenesis” in this new environment [2].

Cancer metastasis remains the major cause of cancer-related mortality, as cancer cells manage to escape the primary tumour, survive the treacherous transit through the lymphovascular system, and eventually form secondary tumour in distant organs [3], [4]. One key step in metastasis is the entry of circulating tumor cells (CTCs) into secondary or distant organ sites to become disseminated tumor cells for subsequent metastasis; however, this step is critically affected by the local microenvironment that CTCs encounter, which determines whether or not tumor cell colonization can happen [5]. Cancer metastasis has continued to confound researchers owing to its complex and not completely determinable, pathological trajectory. The complexity in disease progression arises because essentially cancer is a disease of abnormal cell proliferation, and metastasis involves successfully encountering physiological hurdles, both aspects resulting in a strong evolutionary thrust defining the metastatic cascade [1].

In 2016, Celià-Terrassa and Kang reported that primary tumors are known to constantly shed a large number of cancer cells into systemic dissemination, yet only a tiny fraction of these cells is capable of forming overt metastases [6]. Cancer cells metastatic inefficiency is maintained through a tightly regulated biochemical process such as programmed cell death (apoptosis, anoikis, autophagy, and necroptosis) plays a crucial roles in metastatic processes and also ensures that only few of the transformed cells (cancer cells) migrating from the primary (local) site to secondary (distant) site will give rise to metastases. According to Christopher et al., (2001), apoptotic process is reported to be an early event in metastatic inefficiency [7]. Several secretory and mechanophysical

barrier such as secretion of thrombospondin-1 (TSP1), angiomin, interstitial pressure, haemodynamic force and sheering plays an important role in malignant arrest and in elimination of metastatic cells in the vascular system respectively. In addition, most metastasizing cells are not able evade cell death and arrest resulting from recognition and destruction by cytotoxic lymphocytes such as natural killer (NK) cells, CD4⁺ and CD8⁺ T cells[8], [9]. In addition, the switch-on mechanisms of metastasis suppressor genes (MSGs) such as NM23 [10], KISS1, and NME1 play a crucial role in ensuring metastatic inefficiency [11]. Interstitial pressure, haemodynamic forces and sheering, and induction of malignant growth arrest induced by thrombospondin-1 (TSP1) present in the basement membrane surrounding mature blood vessels [12].

Metastasis is often described as a ‘cascade’ of events, since there are many steps, all of which are interconnected through a series of adhesive interactions and invasive processes, as well as responses to chemotactic stimuli. A metastatic tumour cell needs to successfully complete the entire cascade, and reports from previous studies shows that the vast majority of cancer cells are unable to do so [13], [14], [15]. However, not all malignant cells that migrate from the primary site of the tumour can escape the cascade mechanisms of cell death or arrest that will be encounter during the metastatic process, thus, metastasis is said to be an inefficient process[12].

Understanding Cancer Cells Metastatic Cascade

Metastasis cascade” describe a biochemical progressive steps that occurs during the metastatic process which includes: epithelial–mesenchymal transition (EMT) and breach of the basement membrane barrier; dissociation of tumor cells from the bulk tumor; invasion of the neighboring tissue; intravasation into preexisting and newly formed blood and lymph vessels; transport through vessels; extravasation from vessels; establishment of disseminated cells (which can stay dormant for a prolonged period of time) at a secondary anatomical site; and outgrowth of micrometastases and

macrometastases/secondary tumors [3]. In addition, the creation of a “premetastatic niche” at the target site, before the first tumor cells arrive at this distant location is also an important step [2]. Each stage of the metastatic cascade triggers many physiological barriers to the spread of malignant cells and for the transformed cells to metastasis to the distant site, it must overcome all the biophysiological barriers. Moreover, only certain cells within a heterogeneous tumor population are capable of achieving these steps [16], [17].

Steps in metastasis can be elucidated using five steps which are collectively termed the metastatic cascade [18].

1) *Invasion and migration*: individual cells detach themselves from the primary tumor and invade adjacent, healthy tissue. During this process, several lytic enzymes are secreted which degrade the ECM (extracellular matrix) and, therefore, facilitate migration.

2) *Intravasation*: the intrusion of cancer cells into the blood and lymphatic vessels. After the attachment on the endothelial cells via adhesion molecules, the neoplastic cells secrete proteolytic enzymes which enable them to infiltrate the blood vessel.

3) *Circulation*: the aberrant cell travels via the blood stream and has to withstand the conditions present in the blood. These are toxic for cancer cells due to the high concentration of oxygen and cytotoxic lymphocytes. A selection for particularly resistant and aggressive tumor cells takes place.

4) *Extravasation*: the cells often get stuck in the capillaries of an organ and leave the blood stream by penetrating the endothelium through proliferation and/or proteolytic enzymes.

5) *Colonization, proliferation and angiogenesis*: the neoplastic cell settles at a distant tissue site and builds a secondary tumor. The latter proliferates and induces neo-angiogenesis in order to ensure sufficient vascularization.

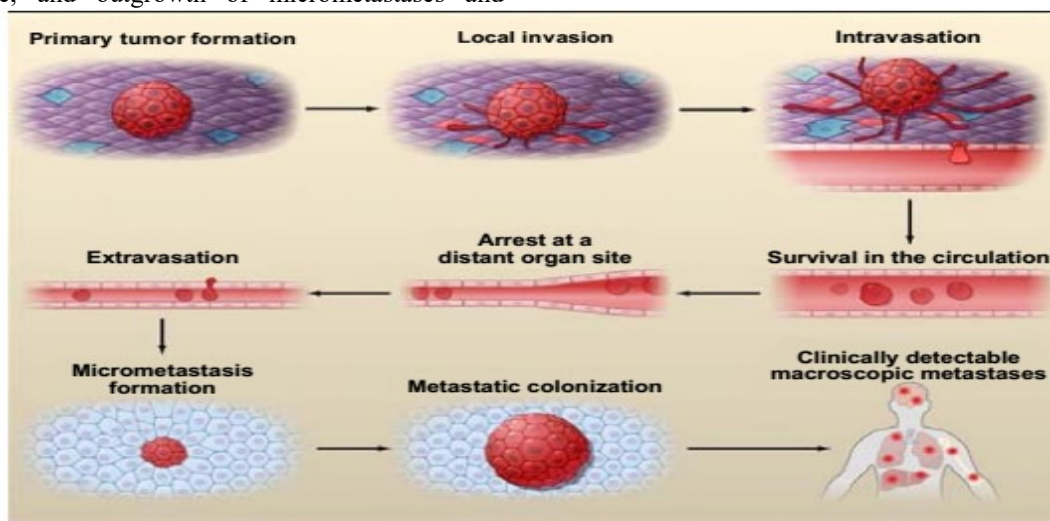


Fig. 1 The steps in Metastatic Cascade [19].

During metastatic progression, tumor cells exit their primary sites of growth (local invasion, intravasation), translocate systemically (survival in the circulation, arrest at a distant organ site, extravasation), and adapt to survive and thrive in the foreign microenvironments of distant tissues (micrometastasis formation, metastatic colonization). Cancer cells are depicted in red [3].

1) *Dissemination of cancer cells and epithelial–mesenchymal transition (EMT):* Local invasiveness involves entry of cancer cells that have resided within a well-confined primary tumor into the surrounding tumor-associated stroma and thereafter into the adjacent normal tissue parenchyma [20]. In order to invade the stroma, cancer cells must first breach the basement membrane (BM), which is a specialized extracellular matrix (ECM) that plays vital roles in organizing epithelial tissues, in part by separating their epithelial and stromal compartments. This phenomenon represents the first step in the metastasis cascade. The cancer cell loses its polarity and there is also down regulation of epithelial proteins, mainly E-cadherin, but also occludin, claudins, cytokeratins or catenin proteins. Cadherins and catenins participate in cell–cell adhesion mechanism [2], [21]. Additionally, cells acquire a spindle-shaped morphology that allow cell migration and induce the production of mesenchymal proteins like N cadherin, vimentin, tenascin C, laminin $\alpha 1$ or collagen type VI $\alpha 1$, as well as various proteinases [22].

The induction of mesenchymal proteins during EMT also promotes invasive and metastatic processes: over expression of N cadherin, for example, induces cell migration, invasion and metastasis. The snail and twist families of EMT mediators also inhibit apoptosis affecting both tumor growth and tumor spreading [23]. Recently, it has been shown that snail members mediate tumor immunosuppression and facilitating metastasis [24]. In addition, twist blocks cellular differentiation can interfere with oncogene induced senescence [25].

2) *Invasion and cell migration:* To invade tissues and vessels, cells must acquire the ability to migrate. The cell migration process starts with the extension of cell membrane protrusions, which is controlled by a continuous cycle of actin polymerization and depolymerization. After adhesion to the ECM via integrin and focal adhesion kinase FAK containing complexes and actin–myosin 2-mediated cell contraction, release of adhesion at the trailing edge leads to cell locomotion. In this process, the cofilin pathway acts as the “steering wheel of the cell” by coordinating membrane protrusion [26]. Similarly, integrin signaling is critical for cell migration and invasion by modulating FAK/SRC signaling and the activity of RHO family GTPases [27].

Once the cancer cells lose contact with the BM during invasion they face another barrier against metastasis: Anoikis (cell death induced by inappropriate or loss of cell adhesion). Anoikis suppression is likely to be a prerequisite for tumor cells to successfully metastasize to distant sites. Consistent

with this, most cell lines established from human tumors contain populations of cells that survive when confronted with lack of adhesion to culture plates [2]. The main cell surface receptors to “sense” adhesion to the ECM and to provide a cell with information about its surroundings are the integrins. Different integrin complexes bind to diverse ECM molecules and respond by triggering an intracellular signaling cascade via focal adhesion kinase (FAK) and SRC family kinases. Integrin activation protects cells against anoikis, similar to several kinases downstream of integrins, including SRC, focal adhesion Kinase FAK and integrin linked kinase (ILK) [28]. Tumor cells often show an altered spectrum of integrin receptors or have high levels of FAK, stimulating proliferation, survival and migration [29].

Tumor cell invasion alone is not sufficient to produce distant metastases; it requires also the transport of malignant cells through blood and/or lymph vessels. It is known that avascular tumors cannot grow beyond a size of 1 mm in diameter [30]. At this stage, passive diffusion of nutrients and oxygen becomes rate limiting for the tumor nodule, which is then forced to enter a state of so called “tumor dormancy”. However, in most cases, tumor vascularization is achieved by sustained angiogenesis (sprouting of new vessels from existing ones), with a significant contribution of bone marrow-derived vascular and hematopoietic progenitor cells [31]. The growth of new vessels is strictly regulated by a delicate balance of angiogenic activators most prominently vascular endothelial growth factor A (VEGFA), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) and angiogenic inhibitors (thrombospondin 1, angiostatin, endostatin and tumstatin) [32], [33].

Under hypoxic conditions the cancer cells promote not only sustained angiogenesis but can also induce and select an invasive and metastatic phenotype and hypoxia-inducible factors (HIF1A, HIF2A) [34]. HIF1A regulates numerous target genes, including many that are involved in angiogenesis (VEGF), cell proliferation and glucose metabolism. HIF1A can promote cell migration and invasion in different ways involving up regulation of the CXCR4 and up regulating lysyl oxidase (LOX) [35]. Furthermore, several EMT mediators, including twist, snail, ZEB1 and ZEB2, are induced by hypoxia and HIF1A in different cancer types [36].

Invasive tumor cells can migrate either as single cells or collectively in the form of files, clusters, or sheets. Collective invasion of tumor cells has been observed also in tumors with incomplete or no EMT. Single cell migration can occur either as a “mesenchymal” migration or in an “amoeboid” form, which is faster and requires no proteolytic ECM remodeling [37]. Many adhesion and signaling molecules, including integrins, CD44 and several Immunoglobulin-domain Cell Adhesion Molecules (IgCAMs), have been implicated in cell migration and tumor invasion [38].

3) *Intravasation, Transport through vessels and Extravasation:* The term intravasation describes invasive

tumor cells entering the lumina of lymphatic or, mainly, blood vessels; process guided by macrophages and involving a paracrine signaling loop relying on the CSF1 receptor (expressed on macrophages) and EGFR (expressed on tumor cells) [39]. To facilitate the intravasation, a range of molecular changes can promote the ability of tumor cells to cross the pericyte and endothelial cell barriers, the microvessels walls' components [2]. As soon as the cancer cells reach the lumina of blood vessels they disseminate widely through the venous and arterial circulation as CTCs (circulation tumor cells). However, while in the hematogeneous circulation, CTCs go through a series of stresses, such as sheer forces caused by the blood flow and the lack of cellular adhesion. As a result, a large number of tumor cells undergo anoikis, eliminating disseminated tumor cells and hampering metastasis [2]. Once located in the microvassels of distant sites, the circulating tumor cells initiate their extravasation, crossing from the vessel lumina into the tissue parenchyma. During this process, integrins and selectins promote the interaction of tumor cells with platelets, leukocytes and endothelial cells, allowing CTCs penetration through the layers of pericytes and endothelial cells that separate vessel lumina from the stromal microenvironment [40].

4) *Formation of Micrometastasis*: When tumor cells extravagate, they encounter a foreign microenvironment formed by stromal cells, ECM constituents, available growth factors and cytokines that usually differs from that one of the primary tumor [2]. In order to survive and form micro metastasis, tumor cells use effective mechanisms to modify the metastatic site properties. According to the "premetastatic niche" model, before the arrival of disseminated tumor cells, the primary tumor releases systemic signals (perhaps lysyl oxidase) that promote a range of changes and convert distant sites into more hospitable environments for the survival of those tumor cells and the formation of micrometastases [41]. Simultaneously, metastatic cells can adapt themselves to the new environment by using cell-autonomous processes. One example of such a mechanism involves activation of Src tyrosine kinase signaling [42].

5) *Colonization of Metastatic Cell*: in metastatic colonization, the majority of disseminated tumor cells suffers either slow attrition over periods of weeks and months or persists as micro-colonies in a state of apparent long-term dormancy. The disseminated cancer cells may be quiescent, with their proliferation at metastatic sites greatly impaired due to incompatibilities with the foreign microenvironments that surrounds them [43]. Moreover, the ability of disseminated tumor cells to escape dormancy and to begin active

proliferation may depend on cell non autonomous mechanisms that are needed to convert foreign microenvironments into more hospitable niches. The outgrowth of indolent disseminated cancer cells may depend on the activation and mobilization into the circulation of bone marrow-derived cells and the subsequent recruitment of these cells to a metastatic site. In some cases, these processes may be stimulated by systemic signals released by carcinoma cells, such as osteopontin (OPN) or SDF-1 [44].

On the other hand, the occult micro metastases may proliferate continuously; however, a net increase in their overall number may not occur due to the effects of a high apoptotic rate. The failure of the occult micro metastasis to initiate neo-angiogenesis has been proposed as explanation for this high attrition rate [43]. The "seed-and-soil" hypothesis of metastatic outgrowth articulated more than 120 years ago is still current [45]. More recently, a number of genes whose expression facilitates the metastatic colonization of breast cancer cells specifically to both lung and brain, have been identified [46], [47]. These genes seem to dictate organ specific metastatic tropism due to their ability to compensate for and overcome incompatibilities between the intrinsic growth programs of the disseminated cancer cells and the demands imposed by the particular foreign tissue microenvironment around them [3].

Therefore, the final step of the invasion metastasis cascade imply that the distinct adaptive programs governing metastatic colonization may number in the dozens, with each determined by both; 1) the identity of the organ site at which metastatic colonization occurs and; 2) the tissue of origin of the disseminating primary tumor cells; in other words cancer cells colonizing the lungs utilize different genetic and/or epigenetic programs than do the same breast carcinoma cells colonizing the bone, brain, or liver [3]. Lastly, the accumulation of genetic and/or epigenetic alterations, as well as the co-option of non-neoplastic stromal cells, cancer cells are capable of completing an intricate, multistep, cell-biological process that culminates in the formation of macroscopic, life threatening growths at distant organ sites [3].

Models Explaining the Molecular and Cellular Mechanisms of Metastasis

(A) *Cell-of-Origin Model*: The normal differentiation programs of the cells of origin from which certain primary tumors are derived may already dictate the altered activity of various metastasis virulence genes (depicted in green). Upon subsequent oncogenic transformation and systemic dissemination, these cells may therefore be capable of completing the process of metastatic colonization [48].

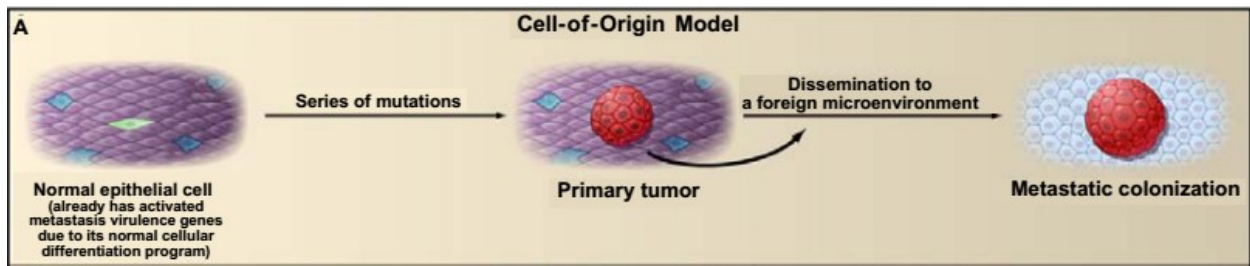


Fig. 2 Scheme illustrating cell-of-origin model [3]

(B) *Partial-Competence Model*: Cells that are only partially metastasis competent (that is tumor cells that have acquired a series of mutations that confer the capacity to disseminate systemically but are initially unable to colonize foreign microenvironments) may arrive at distant organs, where they

then undergo further genetic and/ or epigenetic evolution within these foreign microenvironments to achieve full metastatic competence. Such molecular evolution would likely include alterations in metastasis virulence genes [48].

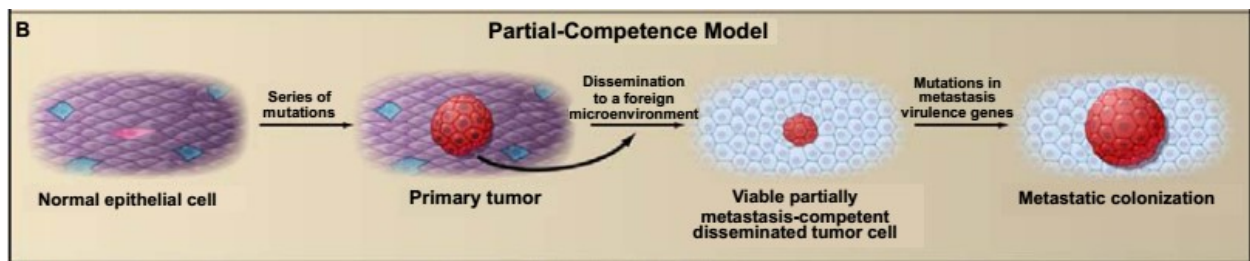


Fig. 3 Scheme illustrating partial-competence model[3].

(C) *Stochastic Model*: Purely by chance, mutations in metastasis virulence genes may accumulate stochastically as “passenger mutations” within tumor cell clones that bear

unrelated “driver mutations” that serve to fuel the clonal expansion of these cells within primary tumors [49].

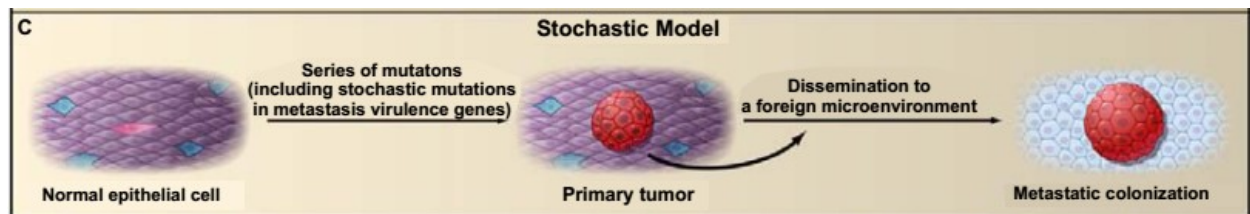


Fig. 4 Scheme illustrating stochastic model [3]

(D) *Tumor Self-Seeding-Model*: The phenomenon of tumor self-seeding indicates that already metastasized cells are capable of re-infiltrating the primary tumor from which they originated. Hence, carcinoma cells present in metastases (which have come to acquire molecular alterations in

metastasis virulence genes via either of the models proposed below, as indicated by the asterisk) may become increasingly represented within their primary tumor of origin (re-infiltrating cells are depicted in blue) [50].

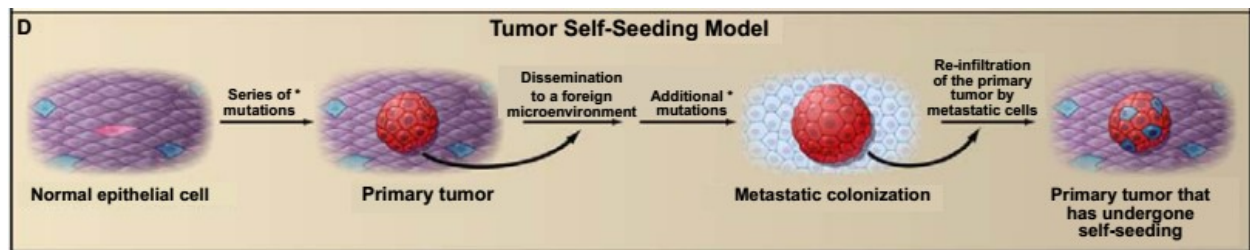


Fig. 5 Scheme illustrating tumour self-seeding-model [3]

(E) *The Parallel Progression Model*: Asserts that quasi-normal epithelial cells (depicted in orange) disseminate very early from pre-neoplastic lesions. Subsequently, these cells undergo molecular evolution at future sites of metastasis

formation. Notably, such sites represent locations where mutations in metastasis virulence genes are now selectively advantageous [50].

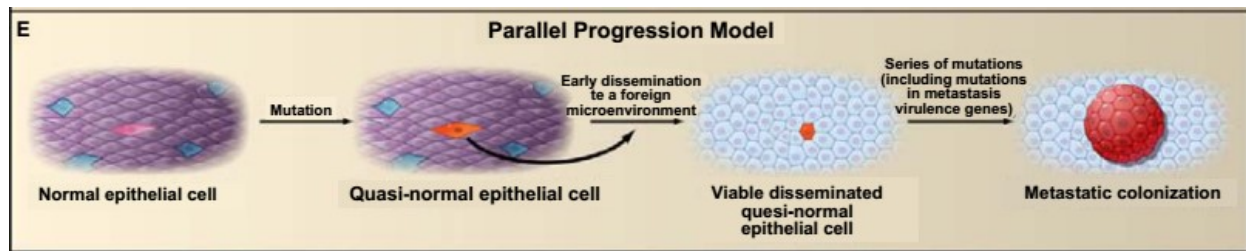


Fig. 6 Scheme illustrating the parallel progression model [3]

II. UNDERSTANDING METASTATIC INEFFICIENCY

The underlying concept reported by Gaorav and Joan, in 2006 has it that metastasis emerges from the somatic evolution of a genetically diversified cancer cell population under the selective pressures of an environment that impose tight rules on cell behavior [16]. Hence, this explains why millions of cells might be released by a tumor into the circulation every day, but only a tiny minority of these cells will colonize a distant organ. The utter inefficiency of the metastatic process implies that healthy tissues display a marked hostility toward invading tumor cells. This facts corroborate with precedent reports maintaining that in a highly evolved organism, homeostatic mechanisms ensure that order is maintained in its tissues. To chalk up metastasis, cancer cells must therefore evade or co-opt multiple rules and barriers that were refined over hundreds of millions of years of organismal evolution. Thus, metastasis is akin to an evolutionary process that involves selection of genetically heterogeneous lineages of cancer cells within the ecosystem of an organism[16].

Experimental reports justifies that metastatic process is inefficient in that very few of the tumor cells released into the circulation develop into metastases; experimental studies have shown that only 0.01% of the tumour cells that enter their bloodstream in animal models successfully form a secondary tumour [13]. More precise studies of metastatic inefficiency have been made with laboratory animals, in which following the injection of known numbers of cancer cells into systemic or portal veins, or directly into the left ventricle, the numbers of resulting tumour colonies in whole organs were counted under the dissecting microscope by histologic examination of serial sections or by bioassays. One of the experiments on metastatic inefficiencies was in the case of different lines of transplantable B16 melanoma cells injected into the tail vein of mice, when the vast majority are arrested at the pulmonary vasculature, injection of 10^5 cells resulted in the subsequent development of medians of 240 (B16.B16) to 4 (B16 wild type) pulmonary colonies, corresponding to inefficiencies of 99.76-99.996%. The most important ultimate route in which cancer cells are disseminated are the blood stream [51], [52]. It was earlier observed that the mere presence of circulating

cancer cells is not synonymous with metastasis, and it was first demonstrated by Iwasaki in 1915 that many if not most of the circulating tumour cells encounters their death within the circulation [53].

Check Point Mechanisms Ensuring Metastatic Inefficiency

The inefficiency of metastasis can be considered from diverse cellular and molecular mechanisms involving the switching on mechanisms of metastatic suppressor genes (MSGs), programmed cell death (apoptosis, necroptosis and autophagy), anoikis (cell death induced by inappropriate or loss of cell adhesion), immune surveillance mechanism ($CD8^+$ T cells, $CD4^+$, natural killer (NK) cells, and non-classical “patrolling” monocytes contribute to prevent tumor metastasis without affecting primary tumor growth), Interstitial pressure, haemodynamic forces and sheering, angiomin and induction of malignant growth arrest induced by thrombospondin-1 (TSP1) present in the basement membrane surrounding mature blood vessels[2], [12], [16].

Induction of metastasis suppressor genes (MSGs): these are special class of genes that are turned off in metastatic cells but, when switch on, they inhibit metastasis without affecting tumorigenicity. At least 12 suppressor genes have been identified to date, beginning with the discovery of NM23 in 1988 [10]. Other MSGs include NME1 – a member of the nucleoside diphosphate kinase family of proteins implicated in cell cycle regulation, KISS1 – a regulator of metalloproteases and a ligand of a G protein-coupled receptor [10], a mitogen activated protein kinase gene (MKK4), and BRMS1 which functions in gap junctions and reduces motility. Each of these genes provides interesting anchor to the spectrum of events thought to be responsible for distinct cellular stages of metastasis.

Induction of programmed cell death malignant cell death: apoptotic induction is an early event geared towards ensuring metastatic inefficiency [7]. Apoptosis was originally thought to be the only form of programmed cell death induced upon expression of malignant cells. However, in the last decade, programmed cell death has expanded to include autophagy and a form of necrosis termed necroptosis (programmed

necrosis). Programmed cell death, especially apoptosis and necroptosis, are natural barriers that restrict malignant cells from surviving and disseminating to distant site [12]. Apoptosis may block metastatic dissemination by killing misplaced cells. Thus, apoptosis serves as an important process for inhibiting metastasis. The success of the metastatic process relies on the ability of malignant cells to evade apoptosis [12].

Disseminating metastatic cells encounters many biophysiological unfavorable conditions beginning from their detachment from the extracellular matrix (ECM), these include; attack by immune cells, hypoxia or a growth factor-lacking environment, that cause increased cellular reactive oxygen species (ROS) production, DNA damage and insufficient energy status as shown in Figure 7 [12]. Low levels of death signals stimulate apoptosis, whereas high levels of death signals often result in necroptosis as shown in Figure 7 below. Due to the activity of the apoptosis, anoikis and necroptosis machineries, most metastatic cells from the primary tumor cannot successfully macrometastasize [12].

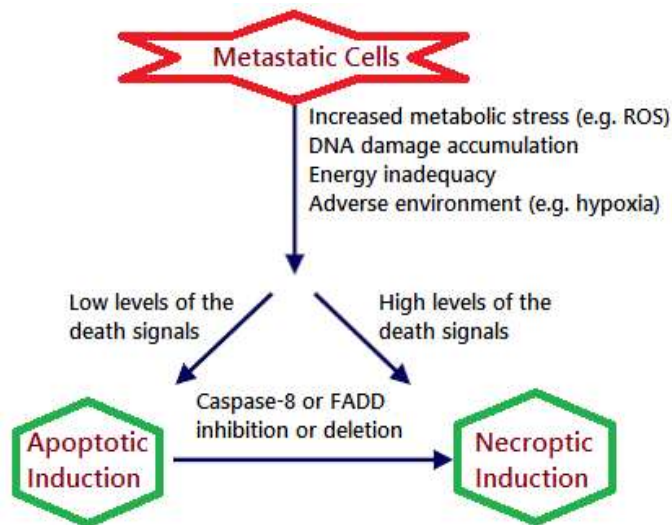


Fig.7 Modified mechanisms between programmed cell deaths against metastatic efficiency [12].

Anoikis Induction: Once cancer cells lose contact with the basement membrane (BM) during invasion they face another barrier against metastasis known as “Anoikis” (cell death induced by inappropriate or loss of cell adhesion) [2]. Loss of cell-cell interaction, cell-matrix interaction and inadequate adhesive substrate in normal endothelial and epithelial cells actively triggers apoptotic response, thus ensuring tissue homeostasis. Therefore, anoikis suppression is likely to be a prerequisite for tumor cells to successfully metastasize to distant sites [54]. Nutrient deprivation and hypoxia, alterations in extracellular adhesions, changes in cell shape during invasion, and exposure to novel stromal microenvironments can all trigger cell death [16].

Induction of angiogenic latency and tumour dormancy: deprivation of migrating malignant cells in the vascular

system due to their size from passive diffusion of nutrient and oxygen halts metastatic efficiency. Tumor cell invasion alone is not sufficient to produce distant metastases, it requires also the transport of malignant cells through blood and/or lymph vessels. It is known that a vascular tumors cannot grow beyond a size of 1 mm in diameter [30]. At this stage, passive diffusion of nutrients and oxygen becomes rate limiting for the tumor nodule, which is then forced to enter a state of so-called “tumor dormancy” [2].

Inefficient vascularization causes the tumour mass to remain constant due to an equilibrium between the cells that are dividing and those which are dying. This is the reason why tumour cells, when forming a micrometastasis, must vascularize it in order to survive, as if this does not occur, it can disappear or enter into a state of latency, a condition in which it will remain until genetic, epigenetic, and microenvironmental signals can activate angiogenesis [2]. In a study of immunodeficient mice who carried a liposarcoma that was able to remain latent for more than 90 days, high levels of thrombospondin (TSP) and angiomin were demonstrated [55]. Thrombospondin or TSP is a glycoprotein of the cellular matrix which in physiological conditions is segregated by the fibroblasts and other cells, such as the endothelial cells [56].

Metastatic cells that have managed to extravasate seem almost invariably destined to either be eliminated from the tissue parenchyma or to enter into a state of dormancy [14], in which they persist in an indolent state as single disseminated tumor cells (DTCs) or as small micrometastatic clusters sometimes for weeks, months, even years [57]. Having traveled far from the primary tumor, disseminating tumour cells (DTCs) find themselves in a new tissue microenvironment that is devoid of the familiar stromal cells, growth factors, and ECM constituents that previously sustained the lives of their predecessors in the primary site. Hence, their inability to continue proliferating and the resulting entrance into a prolonged growth-arrested state may often be attributable to a microenvironment to which these cells are poorly adapted when they first arrive after extravasation. When portrayed in this way, metastatic dormancy reflects a failure of disseminated tumor cells (DTCs) to adapt to and colonize a given tissue [57].

A vast numbers of dormancy-inducing signals found in the microenvironment of certain target tissues have been identified as well [57]. For instance, TGF- β 2, present in high concentrations in the bone marrow and acting through stimulation of TGF- β -RI and TGF β -RIII displayed by disseminating tumour cells (DTCs,) can impose a state of dormancy upon head-and-neck squamous carcinoma cells [58]. Members of the related BMP ligand family have also been linked to metastatic dormancy. BMP7, which can be produced by bone stromal cells, can induce dormancy in prostate cancer cells [59]. Many of these dormancy-inducing cytokines lead to activation of the p38 MAPK pathway; coupled with the absence of mitogenic signals, this has the net

effect of promoting an ERK^{low}/p38^{high} state in DTCs, which leads in turn to arrest in the G₀/G₁ phases of the cell cycle and associated quiescence as shown in Figure 8 [60].

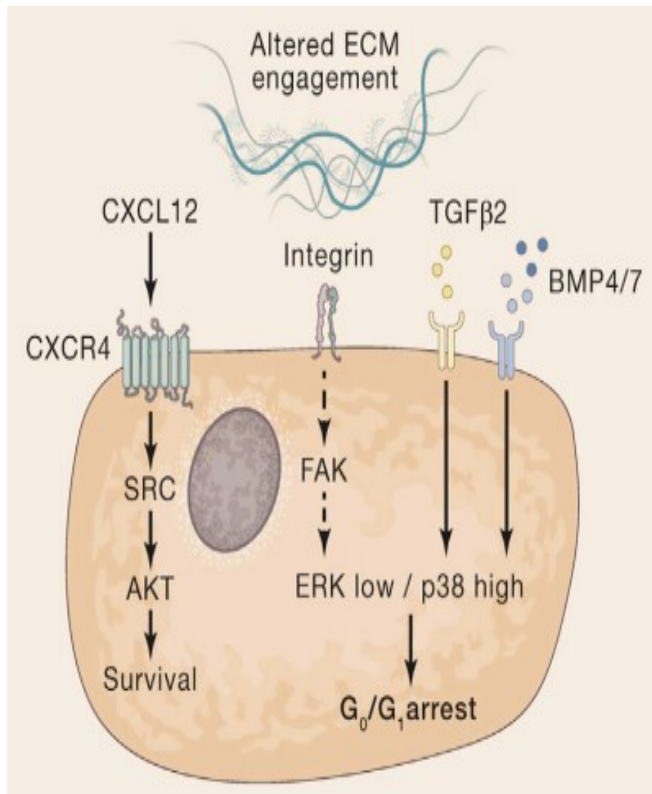


Fig. 8 Model of malignant cell dormancy upon extravasation [57].

Dormant DTCs rely on unique biochemical signaling pathways that sustain their survival and impose programs of quiescence. Signals from the microenvironment, such as CXCL12, can activate SRC and AKT to promote DTC survival. However, reduced integrin-mediated mitogenic signaling, coupled with the actions of certain dormancy-inducing cytokines, enacts a quiescent program in DTCs that is associated with an ERK^{low}/p38^{high} signaling state as shown in Figure 8 [57]. A combined inhibition of both the SRC and ERK pathways by dormancy-inducing agents (ERK low/p38 high, IFN γ , TGF- β 2, TGF- β -R1, BMP) blocks the escape of DTCs from dormancy and thus prevents their subsequent success in metastatic colonization [61].

Immunosurveillance induction: steps in tumor progression and tumor metastasis are halted due to contributory efforts of the inherent immunosurveillance system [5]. The natural killer (NK) cells, CD8⁺ T cells, and non-classical ‘patrolling’ monocytes are the key immunosurveillance agents whose collective effects contribute to prevent tumor metastasis without affecting primary tumor growth [8], [9]. It has been reported that both CD4⁺ and CD8⁺ T cells have been implicated in the control of dormant primary tumor cells through the secretion of IFN γ as shown in Figure 9 [62], [63], in addition, there is also evidence that CD8⁺ T cells can hold disseminated uveal melanoma cells in a dormant state [64].

Thrombospondin-1 (TSP1) produced from mature endothelial cells and deposited in the microvascular basement membrane surrounding mature blood vessels is able to confine DTCs to residence in a quiescent state as shown in Figure 9 [65]. This indicates that the innate immune system is an important component of the dormant niche that effectively forces many cancer cells into a quiescent state [57]. Headley *et al.*, (2016) demonstrated a different process suggested by the observation that antigen-presenting dendritic cells can protect against metastasis [66], thus, implying a role of the adaptive immune system in controlling the growth of metastatic deposits [57].

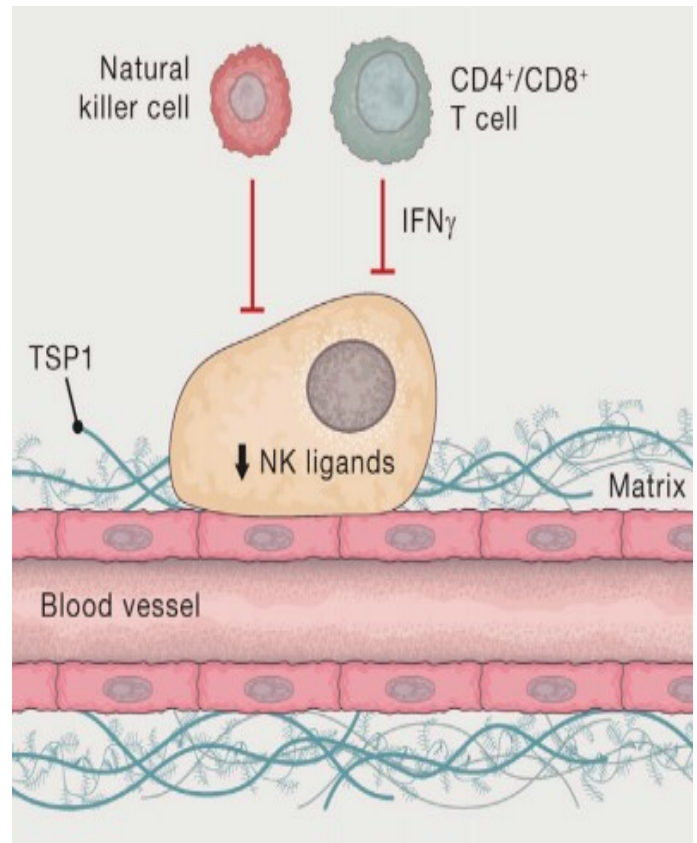


Fig. 9 Immunosurveillance mechanisms against metastasizing cells [57].

Dormant cells can evade detection by NK cells through the repression of NK cell activating ligands and are likely subject to surveillance by the adaptive immune system, which may keep cancer cells in a dormant state through the actions of IFN γ [57]. The tumor-suppressive activities of lymphocytes (T, B, and NK cells) are kept in check against aberrant cells [16].

Mechanophysical activities: Once cancer cells have invaded the blood stream, many of them will die from stresses associated with circulatory passage cells [16]. Variations between vascular tumour size and capillary system results in generating several mechanical and physical stress factors such as interstitial pressure, tension forces, haemodynamic forces and shear against metastasizing cells. Rapid mechanical

lodging in capillaries and association with platelets are likely a prevalent form of tumor cell entrapment and destruction upon circulation and in distant organs [16].

Tumour microenvironment in metastatic inefficiency: Several factors in the tumour microenvironment that limit tumor progression include extracellular matrix components, basement membranes, reactive oxygen species, the limited availability of nutrients and oxygen, and attack by the immune system [16]. The response of tumour cells against these external cues influences, sometimes in dramatic fashion, their metastatic potentials [16]. Each of the steps necessary in producing metastasis, from the arrival of malignant cells to their growth and proliferation in the host organ, is led by the

genetic and/or epigenetic alterations acquired and accumulated during the course of tumour progression [3]. Aberrant hyperproliferation of normal cells and tumour cells are controlled by the cell-intrinsic mechanisms. However, bypass of these cellular restraints, in part fueled by genomic and epigenomic instabilities, is a hallmark of cancer metastasis [16]. The evolutionary conserved local microenvironment provides extrinsic barriers that preserve normal tissue structure and function. These barriers can be broadly classified as chemical, physical, or biological in nature. These extrinsic barriers inhibit the outgrowth of tumors from the primary site to the secondary site as shown in Figure 10 [16].

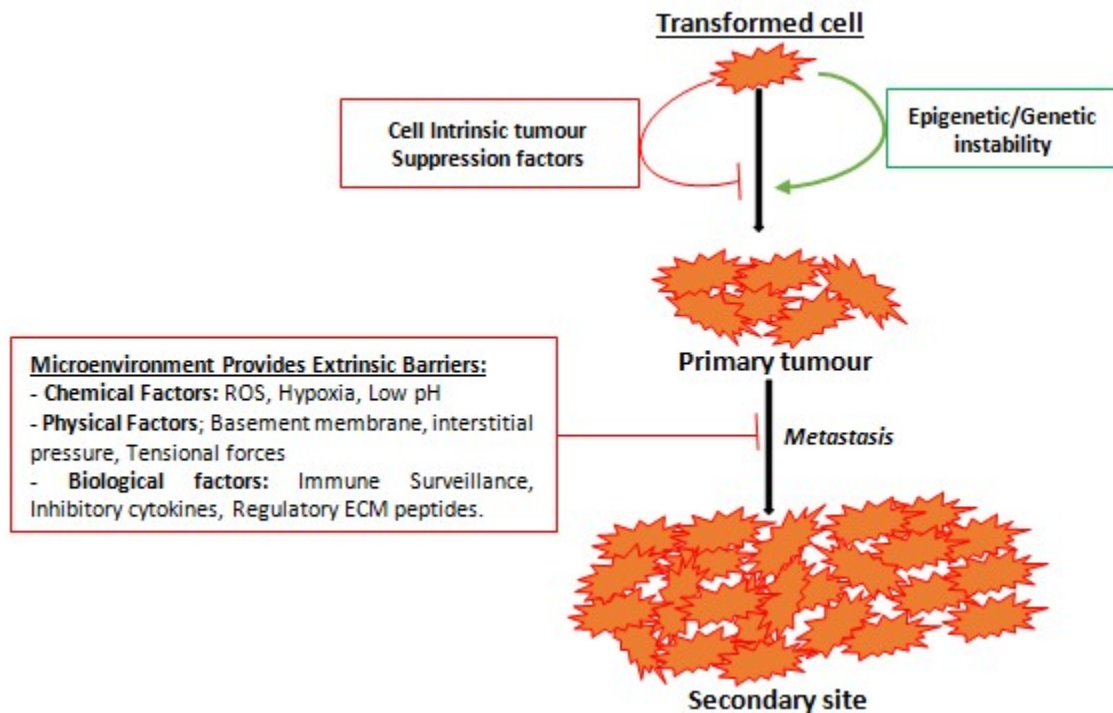


Fig. 10 Modified overview of factors that drives metastatic inefficiency [16].

The quality of the migrated transformed cells (MTCs) from the primary tumour site to the quality of the vascular system in which they progress through and to the quality of the pre-metastatic niche they colonize at the secondary site are the key factors for metastasis rather than the quantity of the MTC from the primary tumour site. Metastasizing cells must acquire suitable functional properties to leave a primary tumor site and arrive into a new niche [67]. The pre-metastatic niche is constituted by the formation of a permissive environment that allows the implantation of metastatic cells and creates a suitable context for the selection of the cells that will be able to survive and thrive in this new soil [67].

The activation of anti-metastatic mechanism against metastasizing cells is the central paradigm upholding metastatic inefficiency. The ability of the transformed cells to acquire chameleonic properties enables them to detach from their primary site and set to progress to a secondary site. However, not all transformed cells are able to metastasize as several anti-metastatic activities are recruited against transformed cells to terminate their progression from the primary tumour site to distant organ as shown in Figure 11 below.

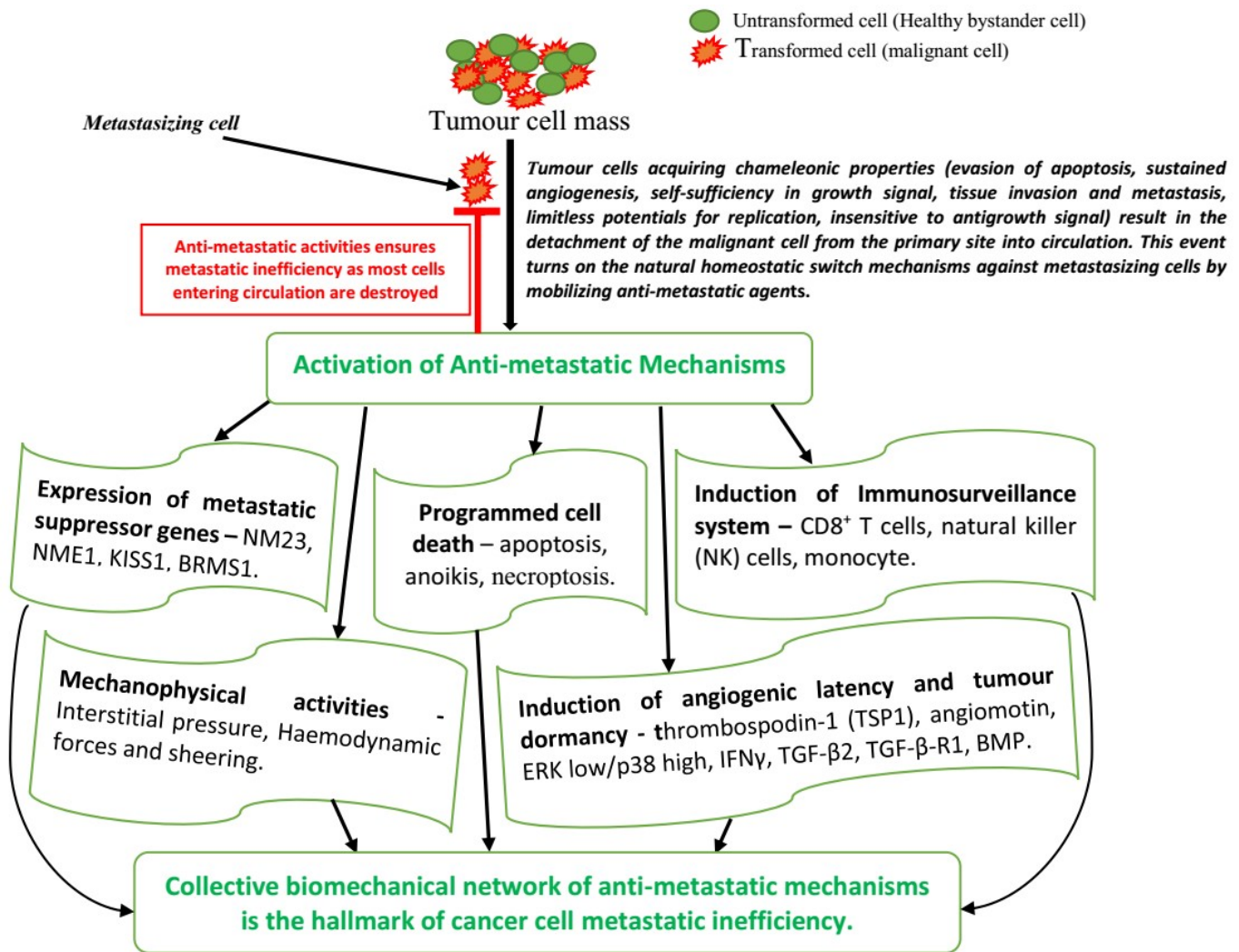


Fig. 11 Biomechanistic activities of anti-metastatic agents against metastatic cells

Tumours at the primary site are known to constantly shed a large number of cancer cells into systemic dissemination, yet only a tiny fraction of these cells is capable of forming overt metastases [6]. It is uncertain at which steps in the process cells are lost, but it has generally been thought that most cancer cells are rapidly destroyed in the circulation [13], either by the immune system (destruction by cytotoxic lymphocytes such as natural killer (NK) cells) [68], [69], hemodynamic forces and sheering [70] and programmed cell death involving apoptosis, necroptosis and anoikis [12]. Myriad of circulating malignant cells can be released from primary tumors site, however, most patients develop only a few metastases, suggesting that metastatic processes (such as invasion, intravasation, extravasation and colonization at distant organs) are quite inefficient but key for controlling metastatic cancer diseases [71].

III. CONCLUSION

Metastasis is an inefficient process as most metastatic cells leaving the primary tumour site do not metastasis to distant organ. The intravasation of metastatic cells from the primary

site of tumour cells during metastasis is inversely proportional to extravasation process and colonization of the malignant cells at the secondary site, that is, most of the tumour cells entering the circulatory compartment to disseminate to the secondary site are engulf, arrested or mopped up by myriads of biochemical intervention including host immunity surveillance (cytotoxic lymphocytes such as natural killer cells, CD8⁺ T cells), activation of metastatic suppressor genes, programmed cell death (apoptosis, anoikis and necroptosis), Interstitial pressure, haemodynamic forces and sheering. However, some viable metastatic cells will enter a growth arrest state induced by angiomin and thrombospondin-1 (TSP1) present in the basement membrane surrounding mature blood vessels which can remain viable and clinically undetectable for extended periods of time and are termed tumour dormant cells. The ability of all the metastasizing cells to overcome all the various forms of cell death and arrest induced by myriads of biochemical processes in the biological system has been proven abortive, hence, metastasis is an inefficient process.

Significance statement

The development of a potent anti-cancer therapy to target cancer cells without affecting normal cells still remains a “holy grail” in the treatment of cancer patient. However, This work elucidate the mechanistic events in the biological system delineating metastatic inefficiency which can offer a new insight that may pave a way for developing a more potent therapy highly specific for malignant cells without affecting the normal cells (healthy bystander cell). In addition, this review will help the researcher to uncover the critical areas of mitigating the menace of cancer in the society.

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