Cancer metastasis - tricks of the trade

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ABSTRACT

Decades of cancer research have unraveled genetic, epigenetic and molecular pathways leading to plausible therapeutic targets; many of which hold great promise in improving clinical outcomes. Metastatic tumors become evident early on and are one of the major causes of cancer-related fatalities worldwide. This review depicts the sequential events of cancer metastasis. Genetic and epigenetic heterogeneity influences local tumor cell invasion, intravasation, survival in circulation, extravasation and colonization to distant sites. Each sequential event is associated with heterogeneous tumor microenvironment, gain of competence, unique population of cancer stem cells (CSCs), circulatory pathway, compatible niche and immune system support. A tight regulation of metastasis-promoting mechanisms and, in parallel, evading inhibitory mechanisms contribute to the severity and site of metastasis. A comprehensive understanding of tumor cell fate as an individual entity, as well as in combination with different promoting factors and associated molecular mechanisms, is anticipated in the coming years. This will enable scientists to depict design strategies for targeted cancer therapies.

KEY WORDS: Cancer; metastasis; tumor cell infiltration; disseminated tumor cells; circulating tumor cells
DOI: http://dx.doi.org/10.17305/bjbms.2017.1908 *Bosn J Basic Med Sci. 2017;17(3):172-182. © 2017 ABMSFBIH*

INTRODUCTION

The past decade has witnessed an immense exploration of the metastatic events in different cancers. Metastasis accounts for 90% of cancer-related deaths in patients with palpable clinical traits, subjective to the cancer type. Colossal development of advanced technologies has enabled scientists to explore potential therapeutic targets that are, presently, in different stages of clinical evaluation [1-3]. In 2011, Hanahan and Weinberg elaborated on the hallmarks of cancer; explaining that cells acquire certain capabilities which are associated with tumor development [4]. The interplay between different factors controls the balance that ultimately facilitates the oncogenic transformation of cells and a possible metastasis, as illustrated in the graphical model in Figure 1. Cancer metastasis has been, in principle, classified into different stages commencing from local invasion, intravasation, survival in circulation, extravasation, and finally colonization and metastasis. Genetic events empowering cells with oncogenic potential, avoiding cell death, rewiring metabolic pathways and dodging the immune surveillance [4].

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Submitted: 03 January 2017/Accepted: 22 January 2017

Malignant cells from the primary tumor infiltrate into the surrounding parenchyma and enter into the circulation by blood vessel intravasation. These disseminated tumor cells (DTCs) travel to distant areas where, upon entrapment, extravasate from the circulation and enter into the target tissue. Upon acquiring specific functional properties, the preliminary micrometastatic mass grows into macroscopic metastasis. This process is also referred to as the colonization of a target organ [5]. The progression of metastasis in a sequential order, from its origin to the infiltration of tumor to different sites and colonization, following a period of latency, is variable among different cancer types. Invading cancer cells acquire distinct cues when targeting different organs, since organs are anatomically and physiologically distinct. [6]. The survival rate of the circulating tumor cells (CTCs) is around 0.2%, and these CTCs are then able to metastasize to target organs [7]. Upon sampling patient blood, it can be observed that CTCs exceed the number of metastatic lesions [8]. A characteristic feature of cancer metastasis is the ability to infiltrate the same or different organ. DTCs are the cells that survive the infiltration of a target organ. It has been reported that approximately 50% of DTCs metastasize to the bone marrow in cancer patients [9]. This points out that competence for invasion in a target organ is not necessarily followed by a similar competence for colonization. Experimental mouse models, injected intravenously or arterially with cancer cells, have shown that a large number

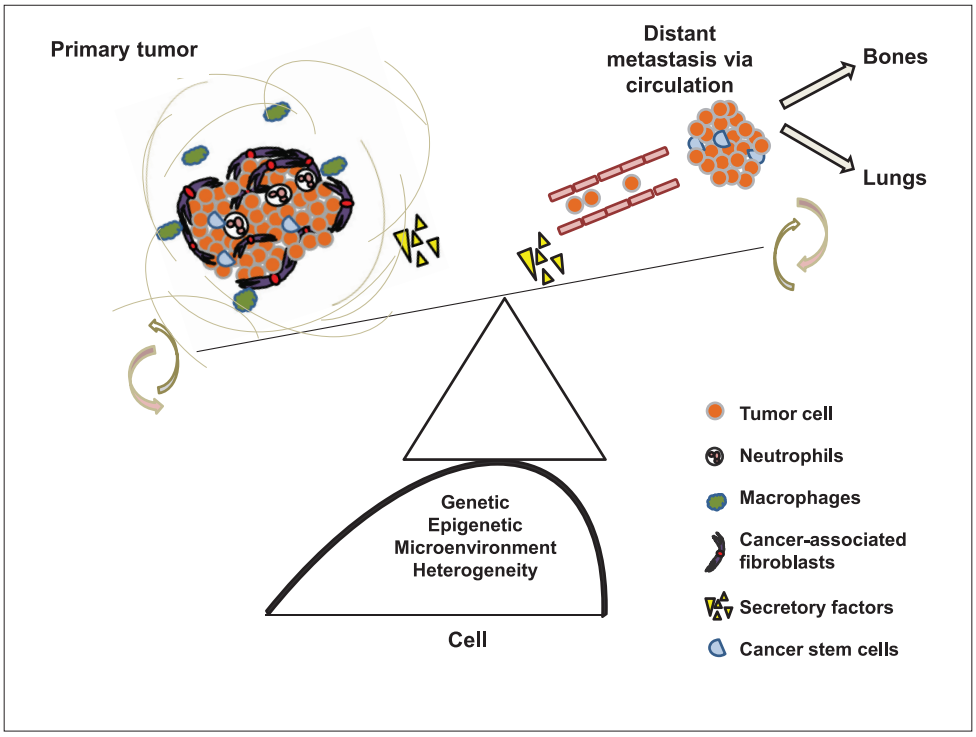

FIGURE 1. The cancerous transformation of any cell depends on breaking the tight regulatory network and acquiring capabilities and compatible microenvironment that facilitate the progression of oncogenic transformations and metastatic potential.

of these cells, reaching the lungs, die within 48 hours; as do the arterially injected cells [10]. A similar loss is evident when breast cancer cells are injected arterially in mouse models, as seen in the case of brain, liver, or bone marrow [11]. Moreover, a similar pattern of inefficiency is reported for CSCs. Most of the breast CSCs residing in the lung are eliminated by apoptosis [12]. This implies that, to an extent, CSCs also rely on the microenvironment that promotes metastasis development leading to colonization.

Reports on the presence of tumors by Greek physicians, between the 5th century and 19th century [13], when the processes of metastasis became evident, showed that cancer metastasizes preferentially to specific organs. This preference is favored by compatible surrounding microenvironment. Following that was the hypothesis by Paget which explained that tumor cells were able to colonize a target organ that provided a favorable microenvironment for these cells [14]. On the contrary, another group argued that site-specific metastasis was governed solely by the circulatory system [15]. The two disparate proposals, nevertheless, indicated that the metastatic potential is evident early on in a diseased state [16,17]. Metastatic cells reach the target organs via circulation, whereas the surrounding microenvironment governs the cell colonization ability [18]. Cancer cells can manipulate the surrounding cells of the tissue to provide support for themselves at the primary tumor site [19,20]. Although it has been reported that, in a glioma patient, DTCs can reach other tissues, generally, the metastasis to a target organ is rare, excluding the cases where the organs were transplanted into immunosuppressed patients [21-23]. Once they leave the primary site, DTCs are more prone to clearance by the immune system [24]. Hence, the survival of these cells is directly associated with their metastatic competence.

Interestingly, the driving force for the tumor has recently been revisited and is now broadly used to encompass all modifications that are either cell autonomous or non-cell autonomous which in any way or at any stage, participate in the tumor evolution. Therefore, it can be stated that the driving force resulting in cell alterations can be either genetic mutations or epigenetic factors. This also includes dysregulation of signaling pathways or mutations in binding factors.

Today, a number of techniques are available for the identification of the factors in each of the above scenarios. However, it should be noted that different driving forces tend to navigate differently at different sites or different stages of tumor development. Hence, genetic mutation (one of the contributors to the metastatic potential) may contribute to tumor progression only at one stage of tumor development [25].

GENETIC INFLUENCE AND TUMOR CELL INFILTRATION

Genes that underlie tumor initiation progression and colonization have been extensively investigated. Acquired advantages attained by oncogenic mutations might be one of the initial dominant factors governing the sequential events preluding metastasis to secondary target site [26]. At the primary site, genes associated with tumor initiation facilitate tumor

cells in the processes of motility, epithelial-mesenchymal transition (EMT) and angiogenesis. This is accompanied with the exploitation of the microenvironment of a target organ. The loss of function of caspase-8 enables tumor cell infiltration, as these cells evade cell death [27]. The genetic makeup affects the susceptibility to metastasis in a particular cancer phenotype [28]. For instance, research on mouse models has indicated that the metastatic patterns in mammary tumor are determined by specific genetic markers [29]. Mounting evidence has demonstrated that the inhibition of oncogenic alterations, i.e., mutations in anaplastic lymphoma kinase in the case of lung cancer and amplification of human epidermal growth factor receptor 2 gene in breast cancer, can efficiently suppress metastatic potential [30,31]. Tumor cells acquire mutations during the progression of tumor, and this facilitates their oncogenic potential. A mutation in von Hippel–Lindau tumor suppressor results in its inactivation which leads to the synthesis of free hypoxia-inducible factor 1-alpha (HIF1α). HIF1α is in turn available for hyperactivation of a number of target genes. As seen in the case of renal carcinoma, elevated hypoxia promotes colonization of tumor cells by hyperactivation of the chemokine receptor (CXCR4) in the bone marrow and lung parenchyma [32,33].

Infiltration of tumor cells can be in two forms, either at the primary site or independently from the primary site. In addition, studies using breast cancer cells indicated the presence of both types of infiltration. Using intravital imaging of breast cancer cells, it has been shown that the transforming growth factor β (TGF-β) cascade can promote the switch between the two forms of cancer cell mobility [34]. EMT has been also shown to promote single cell infiltration by altering the mesenchymal traits and intracellular adhesion [35]. Regulatory epigenetic processes, e.g. post-translational modifications [36,37], as well as transcriptional factors such as slug, snail and twist, can mediate EMT. Reports have indicated that the progression of tumor is evident in human cancer cells after epithelial plasticity is evident [38,39]. Infiltration into the target site requires remodeling of the surrounding environment, which is promoted by the matrix metalloproteinases (MMP) family, and this process, in turn, initiates the release of cytokines such as interleukins (IL) and growth factors (i.e., tumor necrosis factor α [TNF-α] and vascular endothelial growth factor) that further promote cellular growth and survival [40]. Altered vasculature can then lead to intravasation into target organs [41]. Loss of function of a tumor suppressor, cluster of differentiation 82 (CD82), promotes metastasis. Under normal conditions, CD82 interacts with Duffy antigen/chemokine receptor and induces tumor cell senescence [42]. As seen in the case of brain and lung metastases, the secretion of angiopoietin-like 4, TGF-β, cyclooxygenase 2, and MMPs by the tumor cells promotes leaky vasculature, further promoting the invasion of cancer cells [11,43-45]. Tumor cells are also able to communicate with platelets, which facilitates the dissemination and evasion of immune clearance [46]. Upon entering the target site, DTCs start colonization, progressing into the later stages of metastasis [47].

Similarly, loss of function of E-cadherin (CDH1), which is a cell adhesion receptor, results in EMT process, analogous features, where cancer cells acquire invasive capabilities [48-50]. Germline mutations in the *CDH1* gene result in hereditary diffuse gastric cancer [51]. A case study, involving identification of genetic markers involved in cancer metastasis, reported Ras, Src and Wnt as the examples of oncogenic signaling pathways found to be consistently dysregulated in a metastatic phenotype [52]. It has also been observed that dysregulation of developmental pathways contributes to dissemination and infiltration of target organs. As seen in a breast cancer model, where EMT and infiltration are fostered by the overexpression of twist transcription factor that regulates embryonic morphogenesis [53]. Cooperative relation between mesenchymal stem cells and neuroendocrine in promoting metastasis of the later has been shown. This could be due to their ability to manipulate the Ras signaling, enhancing heterogeneity within the tumor [54].

TP53, a tumor suppressor protein, plays an important role in cell growth and apoptosis. TP53 has been identified as a master regulator of metastasis, as it controls the transcription of genes involved in the tumor initiation, progression, and metastasis [55]. TP53 significantly contributes to metastasis considering that around 50% of human cancers have altered TP53; either mutated or with loss of function. Studies have shown that the loss of TP53 facilitates cell migration due to modifications of their polarity and morphology [56]. Mammary epithelial cells with a loss of TP53 have shown an increase in EMT, leading to an increase in stem cells and developing of a tumor [57]. CD44, a breast CSC marker, is upregulated in the case of TP53 loss, promoting tumor cell progression [58]. TP53 controls the transcription of plasminogen activators which are responsible for the degradation of ECM and invasiveness of cells. As seen in breast cancer, loss of TP53 stimulates cell invasion, leading to metastasis [59].

TUMOR CELL SURVIVAL

Several pathways, including the Akt pathway, have been also shown to promote survival and metastasis of DTCs, at various sites. Once cell-matrix interaction is hampered, cells are subject to apoptosis known as anoikis [60]. However, tumor cells can evade this checkpoint and enter the circulation to reach distant target organs. These cells can leave the primary site either as single entities or in the form of clusters. A number of studies have shown that specific cancer cell clones integrate

into each other to promote collective survival and metastatic potential [54,61-63]. Clusters of CTCs have been reported to be more competent in their ability to metastasize as compared to single cells [64]. Such clonal seeding has reportedly been observed in prostate cancer patients [65]. The mouse model has shown that these tumor cells can infiltrate target organs, following the formation of cellular aggregates. The formation of cellular aggregates is initially facilitated by tropomyosin receptor kinase B, a tyrosine kinase receptor, upon activation of the PI3K/Akt pathway [60]. The activation of Akt signaling through Src kinases has been shown to promote tumor cells survival, as demonstrated in the case of bone marrow cancer [66]. Once in the blood stream, the cells are challenged by a number of factors including the innate immune system. The cells associate with platelets, which is one way of avoiding their removal [67]. An example of another strategy is increased dependence of melanoma cells on NADPH-producing enzymes of the folate pathway. This is a mechanism in which cells avoid oxidative stress by inducing reversible metabolic changes. In addition, inhibition of the folate pathway hampers metastatic potential of the tumor cells, as seen in mouse models [68]. DTCs are associated with macrophages due to aberrant expression of vascular cell adhesion molecule 1 (VCAM1). This is seen in case of breast cancer cells where a lung relapse is seen as the overexpression of VCAM1 favors these tumor cells to infiltrate the macrophage-rich lung environment through activation of the Akt pathway mediating prosurvival cues for the DTCs [69]. To finally reach the target organ, CTCs depend, to a certain extent, on the circulatory system of the body. CTCs are initially entrapped within the capillary vessel before extravasation to the target organ. Body's blood circulatory network defines the initial capillary ground that the CTCs confront. The venous circulation flows to the right ventricle and into the lungs in most organs whereas it flows into the liver via the gut. As the result of this circulatory organization, CTCs that are transported to the lung and liver contribute to the development of metastasis in these organs [70].

CTCs integrate into the blood vessel which facilitates their attachment to the vascular endothelium, and their proliferation. This eventually leads to rupturing of the vessels [71]. Alternatively, these CTCs extravasate through the ruptured vessels [7]. In addition, the structure of the vessels contributes to the extravasation. In the case of the liver and bone marrow, the capillaries are lined with fenestrated endothelial cells along with a discontinuous basal lamina that promotes extravasation. This process has been shown to promote high rates of metastasis in the liver and bone [6,72]. In contrast, the capillary bed of the lungs is lined with endothelium and basement membrane strengthened by tight junctions [6]. In the case of the brain, the vessels are strengthened by pericytes and astrocytes forming the blood-brain barrier [73].

ADAPTATION

Once the tumor cells have infiltrated into the target organs, the next step is to adapt and to colonize the microenvironment of the organ. The tumor cells that lack appropriate signals for interaction will be subject to growth arrest or enter into a state of dormancy [47]. Recurrence is seen in the case of breast cancer, where approximately 62% of deaths are accounted for after a 5-year survival [74]. This signifies the importance of understanding the mechanisms governing dormancy in DTCs. Dormancy can be classified into three categories; cell dormancy is where internal and external cues dictate individual or a small number of DTCs to enter a state of quiescence, angiogenic dormancy where altered vascularization hinders tumor mass proliferation and immune - mediated dormancy that induces cytotoxicity and keeps the tumor mass in check and prevents proliferation. DTCs that enter cellular dormancy can be characterized as being in a reversible quiescence state [75], as single cell-based studies have indicated a lack of proliferation markers in these DTCs [76,77]. Stress, induced in cancer cells by serum deprivation, results in inhibition of the PI3K pathway which leads to cells entering the state of quiescence and the process of autophagy [78]. A specific kinase, dual-specificity tyrosine - phosphorylation-regulated kinase 1B (DYRK1B), has been shown to induce the quiescence. This has been observed in pancreatic and ovarian cancer cells, where the inhibition of proteins involved in the $G_0/G_1/S$ cycle (e.g., cyclin D1 and p27) induces the quiescence [79,80]. Alternatively, DYRK1B also contributes to cell survival, however, blocking DYRK1B kinase leads to loss of viability of pancreatic cancer cells that are in quiescence [79]. DTCs have been shown to undergo a variable period of dormancy after accustoming to the new microenvironment, as reports have indicated [81,82]. The microenvironment receiving DTCs can be either permissive or restrictive to the induction of dormancy, as explained elsewhere [83,84]. One example of a dormancy-permissive microenvironment is where bone morphogenetic protein 4 (BMP4), BMP7 and TGF-β2, among other factors, contribute to the state of dormancy of hematopoietic stem cells (HSCs) in the bone marrow. These factors have also been reported to cause residual disease dormancy in many types of cancer [83,85-87]. A microenvironment that restricts the state of dormancy was observed in mouse bone marrow, where metastatic breast cancer cell lines escaped dormancy upon the upregulation of VCAM1. Aberrant expression of VCAM1 induced DTCs to bind to $\alpha4\beta1$ integrin-expressing osteoclasts, which eventually lead to bone metastasis [88].

Tumor cells are also suppressed by other factors, e.g. BMP, that is expressed in the lung stroma. Mouse models have shown that breast cancer cells, with a gain of function of DAN domain family member 5 (*DAND5*) associated genes, result in

a metastatic relapse to the lung. This is because *DAND5* has shown to be an antagonist for BMP, hence facilitating metastasis of DTCs, from a state of dormancy to colonization [87]. Dormant tumor cells, with a fibrotic metastatic abrasion, can exit the dormancy by binding to fibronectin, which is abundant in such environment [89]. Dysregulation of integrin signaling increases the levels of cell division control protein 42 (CDC42) which enhances the flux of mitogen-activated protein kinase 1 signaling [75]. This mechanism leads to the activation of endoplasmic reticulum-associated stress response which favors the dormant state of tumor cells [90,91]. Tumor cells within a metastatic lesion are subject to a markedly different microenvironment as compared to the primary tumor site. The cancer cells can manipulate the stromal cells in the surrounding and positively contribute to the dissemination and modulating a favorable tumor microenvironment at the primary tumor site [19,20]. Recent research in this field indicated that specific mechanisms prepare the primary tumor site before arrival of DTCs [18].

Different organs in the body, e.g. brain, lung, breast or liver, harness a particular microenvironment. This, in turn, induces specific selective pressures on DTCs, before their colonization of a particular organ. This also implies that DTCs will acquire specific cues and functional properties that will promote their metastasis to a specific target organ. For example, prostate cancer cells preferentially metastasize to the bone as compared to any other distant organ. It has also been reported that, for these cells, the bone is the main site of relapse. This further emphasizes the role of organ-specific metastasis [6]. Along with differentiated, postmitotic cells, tumors also contain small populations of CSCs. These CSCs, along with the renewal potential, can withstand chemical and electromagnetic attacks. The small population of CSCs that survives exogenous insults can contribute to local recurrences in the case of solid tumors [92]. Niches with active signaling pathways, such as the Wnt and Hedgehog pathways, facilitate stem cell proliferation, renewal, and differentiation [93,94]. Studies have also indicated that niches contribute to tumor development by providing necessary signals to mutant stem cells for tumor initiation [95,96]. EMT induction is facilitated with the help of tumor stromal-derived factors such as TGF-β and Wnt signaling cascades (canonical and non-canonical) [35]. This results in the induction of EMT transcriptional factors which further leads to the expression of components of snail, twist and others, which promote EMT by upregulating mesenchymal genes [97]. In many different tissues both, canonical and non-canonical signaling cascades induce EMT along with inducing stem cell properties. Shifting and balancing between CSC and non-CSC characteristics is possibly governed by the interplay between paracrine signaling from the tumor stroma and the autocrine signaling from the carcinoma itself [97,98]. CSCs that reach distant places are nourished by the local niches ensuring their survival and proliferative potential [99]. Prostate carcinoma stem cells target niches of hematopoietic stem cells and contribute significantly to bone metastasis [100]. CSCs have been shown to be the drivers of metastasis in human pancreatic cancer, where a study indicated that the loss of CSCs in pancreas carcinoma cell lines disrupted the tumorigenic potential of the cell line [101].

METASTATIC LATENCY

Metastatic latency is the time span between organ infiltration and colonization. Although metastatic latency is significantly relevant to the metastatic events, little information is available to date, due to the scarcity of experimental models investigating the latency period [102]. This period is determined by the competence of infiltrated tumor cells and is subject to different physiological constraints. In addition, this process is variable in different cancer types. The ability of tumor cells to colonize after decades of dormancy, as seen in the case of breast and prostate cancers, suggests that, in such cases, competence can be acquired over a long period of time [103,104]. It is also suggested that during the long period of latency, DTCs and their microenvironment evolve, which is a prerequisite for the colonization [6]. Contrary to requiring longer periods of latency, DTCs promptly acquire competence as seen in the case of lung cancer [105]. However, in colorectal carcinoma, the cells acquire genetic mutations over a long period of over 30 years within the primary tumor [106]. Then, following the acquisition of invasive competence is rapid metastasis into the target organ, without a period of latency. In the case of colorectal carcinoma, in 80% of the patients, DTCs use the mesenteric circulation to metastasize to the liver [107]. A study in mice has shown that inhibitors of differentiation 1 and 3 (ID1 and ID3) promote DTC proliferation into the target lung parenchyma [45]. Bone metastasis is an example where organ-specific colonization has been extensively investigated. Upon leaving the period of latency, breast cancer cells undergo a series of events. The cells gain the competence to secrete osteoclast-activating factors, including TNF-α, IL-11, and IL-6. These factors consequently result in the release of receptor activator of nuclear factor-κ β ligand, which promotes osteoclast formation. These osteoclasts initiate the release of factors such as TGF-β and BMPs from the bone matrix, which stimulates osteolytic metastasis of DTCs [6,33]. In the case of breast cancer cell metastasis, a set of genes, i.e., *IL11*, *CTGF*, *CXCR4*, and *MMP1*, was found to promote the colonization in the bone [33]. Apart from the above mentioned factors, numerous other parameters determine the ultimate fate of tumor cells, from CTCs to DTCs to colonization. In addition, in the early stages of tumor development,

genetic and epigenetic factors determine the fate of individual tumor cells, as well as the fate of groups of cells, as proposed previously [25].

METASTATIC COUNTERPARTS - IMMUNE SYSTEM

Sequential acquisition of metastasis is a complex process that progresses through the association between carcinoma cells and the cells of the immune system (neutrophils, macrophages, etc.), components of the tumor stroma, and proinflammatory cytokines. Remodeling of tumor microenvironment has been associated with the oncogenic potential of cells. The *Myc* gene has been shown to facilitate angiogenesis and tumor cell dissemination by recruiting mast cells into the tumor microenvironment [108]. CXCL8/IL8 signaling promotes neovascularization which is mediated by the *Ras* oncogene [109]. Immune suppression within a tumor is shown to be mediated by chemokines and other cytokines (e.g., CCL2, CCL20, CXCL5, CXCL12, TNF-α, TGF-β, IL-1β, IL8, IL10) that are secreted by tumor and immune cells [110]. Tumor cells have also been shown to evade the immune response by the activation of NF-κB and STAT3 signaling which blocks dendritic cell maturation [111,112]. The NF-κB signaling contributes significantly to cancer metastasis and associated inflammation [113]. The gene expression of proinflammatory cytokines (IL-1 and TNFα), chemokines (IL-8 and MIP-1α), intercellular adhesion molecules (ICAM and VCAM1), and certain growth factors is governed by the activation of NF-κB. Hence, its activation ensures that inflammatory signals are maintained within a tumor microenvironment [114]. Mast cells, neutrophils, and macrophages have been reported to secrete proteases which facilitate ECM remodeling [115]. The survival of CTCs in the circulation is essential for establishing metastasis in target organs. After the activation of NF-κB and STAT3 signaling, the secretion of TNF-α, IL-6 and epiregulin is induced in inflammatory immune and cancer cells within the tumor microenvironment, which are further released into the blood and facilitate CTCs survival in the circulation [6]. A number of studies support the role of CAMs in the progression of metastasis and attachment to endothelial cell walls at distant sites [116]. Tumor cells with VCAM1 expressed on T cells bind to α4β1 (VCAM-1 is a α4β1 ligand), which facilitates migration of lymphocytes and prevents their infiltration into the tumor tissue [117]. Macrophages and pre-osteoblast cells with α4β1 expression can also metastasize to the bone and lung, due to the interaction between VCAM1 (expressed on tumor cells) and α4β1 [69,88]. Breast adenocarcinoma metastasis to the lung has been shown to be promoted by immature myeloid cells in mouse models. These cells reduce interferon-gamma secretion and, in parallel, induce pro-inflammatory cytokines secretion in the pre-metastatic environment. In addition, the myeloid cells also promoted the expression of MMP9, which contributed to the remodeling of the vasculature in the lung [118].

EPIGENETIC CONTRIBUTION

Epigenetics describes molecular processes in which the expression of genes is modified without the alteration of DNA sequence. These modifications are characterized as being reversible and heritable, and play an important role in various processes, including differentiation mechanisms of stem cells (adult and embryonic) and possibly in determining the function of CSCs [119]. Mechanisms that control tumor initiation, stem cell characteristics, and chronic inflammation have been reported to be regulated by the epigenome [120,121]. A genome-wide methylation analysis of paired primary colorectal cancer and liver metastasis samples showed differences in DNA methylation patterns [122]. An interesting study has shown that the promoter region (containing CpG islands) of latexin gene (a negative regulator of HSCs) is hypermethylated in skin cancer cell lines as well as in other cancers [123]. Histone deacetylases (HDACs) remove acetyl groups from histones, and have been reported to regulate cancer initiation and progression. A report indicated that a paired loss of mono-acetylation of H4K16 along with trimethylation of H4K20 could be regarded as a hallmark of cancer cells [124]. Numerous experiments have shown that HDACs are responsible for the repression of transcription of genes, by decreasing histone acetylation. Some of these genes, that can facilitate cancer cells either alone or in combination with other factors, include differentiation factors, inducers of apoptosis, and tumor suppressors [125,126]. A cyclin-dependent kinase inhibitor, p21, has been shown to be repressed by HDACs, and the overexpression of HDACs in different cancers has been associated with the corresponding repression of p21. The overexpression of HDAC1 has been observed in prostate cancer cells [127], while the overexpression of HDAC2 has been detected in gastric carcinoma [128]. This signifies the role of HDACs in cancer progression [126]. Cancer cell invasion leading to metastasis is also evident in the case of class 1 HDACs, since these are responsible for regulating E-cadherin, where the loss of E-cadherin results in the loss of cell adhesion, ultimately influencing metastasis progression [129]. Epigenetic inactivation of the inhibitors of the Wnt/β-catenin signaling cascade has also been reported to contribute to tumor metastasis [130]. In addition, studies on human cancer have shown that the hypermethylation of the promoters of Wnt antagonists, such as SFRP and DKK3, contribute to their dysregulation [131,132]. Similarly, the inactivation of Wnt proteins, such as Wnt7A and Wnt9A, by epigenetic promoter methylation is

seen in the case of pancreatic cancer [133]. Epigenetic mechanisms contribute significantly to the numerous cellular processes which are central to physiological signatures evident in normal as well as malignant conditions. Further understanding will pave the way to elucidate therapeutic options in treatment of cancers [130].

LOOKING AHEAD

At present, available technologies give us an understanding of tumor biology and potential molecular targets. Humanized mouse models have been a key source for providing information regarding molecular aspects of tumor biology, however, due to limited genetic variation these tumors are homogenous. In addition, related ethical issues represent some of the limiting factors. Some areas that will possibly govern research platforms in this filed in the coming years are described as follows. Single-cell genome sequencing technology can be used to address the heterogeneous nature of tumors and to identify mutant alleles. The mutational status of a single cancer cell can provide clues about their evolution. Interestingly, it can also provide an insight into the heterogeneity of a tumor cellular population [25]. Imaging techniques using positron emission tomography and magnetic resonance imaging have also shown promise in improving the clinical outcome [134,135]. Advancements in the area of protein and RNA expression techniques have contributed to the understanding of heterogeneous nature of tumor cells. CyTOF mass cytometry can localize around 32 proteins within a cell [136] and, in parallel, the resolution can extend to subcellular level [137]. *In situ* sequencing techniques are currently being explored for shedding light on the RNA content of cells [138]. When it comes to identifying epigenetic markers, chromatin immunoprecipitation (ChIP) in combination with ChIP sequencing can be a modest choice. Whole-genome bisulfite sequencing technology can provide a comprehensive picture of the methylation patterns [139]. However, it is evident that only synchronized efforts using multiple technologies, targeting a specific site for different markers simultaneously, can accurately determine their respective roles. Technological advancements are being explored to elucidate metastatic events in different malignancies. It is anticipated that more insight into these crosstalk of the molecular events will be available in the near future.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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