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Chapter

Stromal Cells and Extracellular Vesicles

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Abstract

Stromal cells are stem cells in the bone marrow microenvironment that can 'talk' with neighbouring and distant cells within the bone marrow microenvironment. Stromal cells propagate this intercellular communication via cytokines, growth factors as well as small extracellular vesicles. The interaction between stromal cells and the haematopoietic stem cells, is crucial in the regulation of haematopoiesis. Aberration in this regulatory process will lead to the development of various diseases, including cancer. These stromal cells also play important role in the patient's response to cancer therapy. As a result, these stromal cells may be crucial in the development and metastasis of cancer within the bone marrow microenvironment. In this chapter, we will explore the role of these stromal cells in carcinogenesis and cancer metastasis.

Keywords: stromal cells, extracellular vesicles, cancer, metastasis, tumour, microenvironment, bone marrow, therapy

1. Introduction

The bone marrow (BM) consists of multilineage cell types, most especially the haematopoietic and mesenchymal lineage. The interaction between the haematopoietic and mesenchymal cell lineages are crucial in the maintenance of haematopoiesis [1, 2]. As a result, BM is the major site of haematopoiesis, which is the lifelong process of blood cells formation. Within the BM microenvironment or stroma, the haematopoietic and mesenchymal progenitor cells give rise to different cells such as immune cells, osteoclasts endothelial cells, stromal cells (mesenchymal stromal cells; MSC) and nerve cells [1, 3].

These stromal cells are characterised *in vitro* by the International Society for Cellular Therapy (ISCT) by three main qualities; (i) ability to adhere to plastic in standard culture conditions; (ii) expression of CD105, CD73 and CD90 surface molecules but lack expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19, and HLA-DR proteins; (iii) multilineage differentiation potential into osteoblasts, adipocytes, fibroblasts and chondroblasts [4, 5] (Chung et al., 2021). These stromal cells offer haematopoietic support, immunomodulation, and bone remodelling via cell-to-cell contact and/or secretion of soluble factors.

During carcinogenesis, the BM stroma goes rogue and enables cancer cells to recruit supporting cells from the tissue stroma needed to promote critical steps in

tumour formation and thus, constitute a vital cog of the tumour microenvironment (TME) [5, 6] (Chung et al., 2021). These stromal cells are recruited into the TME via secretion of different biomolecular factors such as cytokines, extracellular vesicles (EVs), chemokines, and growth factors. These stromal cells play important roles in all steps of cancer metastasis such as extracellular matrix (ECM) remodelling, migration, invasion, intravasation, circulation, survival, extravasation, and colonisation of distant secondary tumour sites [7–9].

Metastasis refers to the process of dissemination of cancer cells from its point of origin (primary site) to a distant disconnected part of the body, forming macroscopic secondary foci which constitutes a metastatic cancer [10, 11]. Metastasis was coined from the two Greek prefixes "meta" (alteration or change) and "stasis" (an equilibrium state), to represent both a process and its outcome. Despite the advances in cancer treatment, evidence from clinical experience and biologic inferences, show that metastasis is responsible for about 90% of cancer morbidity and mortality, with over two-thirds (66.7%) of deaths originating from solid tumours [12]. Metastasis is one of the hallmarks of cancer have been shown to occur as a complex, sequential but interrelated cell-biological events called the invasion-metastasis cascade [13].

Depending on the tumour type, stromal cell composition within the tumour microenvironment often varies and usually includes mesenchymal stem/stromal cells, pericytes, fibroblasts, adipocytes, vascular endothelial cells, stellate cells, and immune cells such as macrophages, T-cells, and natural killer (NK) cells [14–16]. Once recruited, these stromal cells undergo tumoral education and transform into tumour stroma. These damaged stromal cells are also vulnerable to cancer aggression either via direct contact with each other, through gap junctions thereby resulting in transfer of material from stromal cells to cancer cells [8, 14–16]. These lead to promotion of tumour growth, angiogenesis, proliferation, invasion, metastasis and chemoresistance once recruited to the tumour microenvironment [17].

2. Stromal cells and cancer metastasis

Normally, cells in the human body undergo continuous cellular division to ensure proliferation and differentiation of cells, and removal of damaged/worn-out cells (apoptosis) to ensure balance in the cellular system. Cancer arises when there's uncontrolled growth and/or proliferation of cells in the body without apoptosis. Cancer can emanate anywhere in the human body, and these cancer cells can be benign or malignant. In addition, cancer cells can metastasize or spread into, or invade nearby tissues and can travel to distant places in the body to form new tumours [18, 19].

Cancer cells spread either by invasion of nearby tissues or by movement through the lymphatic and blood vessels. Although different cancers are more likely to spread to downstream organs and lymph nodes close to its primary sites than others, most common metastatic areas include the liver, lung, and bone [18, 19]. Most of the cancers that separate from the original tumour do not survive as they also require the capacity to adhere to the blood or lymph vessels, grow and thrive in the new site as well as evade the attacks from the immune system [19].

However, it is noteworthy to mention that not all cancer cells are metastatic and not all cells within the metastatic tumour have the potential to metastasize [20]. The essential hallmarks of metastasis can be difficult to ascribe since they are superimposed by that of cancer itself, however, these five qualities have been reported

and includes: dissemination (detachment) and invasion, intravasation, circulation, extravasation, and colonisation [11, 21].

3. Endothelial cells

Endothelial cells are crucial in the promotion of cancer cell migration, invasion, and metastasis. During tumorigenesis, gaseous exchange and nutrient transport occur by passive diffusion however an increase in the volume of the tumours (1–2 mm^3) leads to insufficient oxygen and a build-up of metabolic waste in the tumour microenvironment [6, 15, 22, 23]. This makes the tumour microenvironment to become hypoxic and acidic thereby highlighting a need for the tumours to develop their own blood supply to overcome this.

The vascular endothelium, a thin layer of endothelial cells, aids in orchestrating the separation of circulating blood from tissues, delivery of water, oxygen and nutrients, movement and adhesion of leucocytes, and formation of blood vessels within the tumour microenvironment [6, 22, 23]. The vascular endothelium is highly organised and hierarchical in structure, and this enables the interaction between stromal and non-stromal cells to provide support and stability for the blood vessels.

Tumours co-opt existing blood vessels and induce growth of new blood vessels by a mechanism known as vessel sprouting [6, 23]. Abnormal sprouts are characteristic of the tumour vasculature along with intercellular gaps and no hierarchical arrangement. These vascular endothelial cells within the tumour microenvironment interact with tumour cells and other stromal cells to promote tumorigenesis and metastasis.

The hypoxic tumour microenvironment leads to expression and activation of hypoxia-inducible factors (HIFs) that co-ordinate cellular response to low oxygen levels. These HIFs then instruct the endothelial cells to secrete and release proangiogenic factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) thereby initiating vessel sprouting [6, 22–24]. These proangiogenic factors, especially VEGF then promote vascular permeability and angiogenesis by stimulating the migration of endothelial cells to form new blood vessel lumen in an autocrine and paracrine fashion. Activation of VEGF receptors (VEGFR) on endothelial cells also activate several downstream signalling pathways including the mitogen-activated protein kinases and extracellular signal-regulated kinases (MAPK/ERK) and phosphatidylinositol-3 kinases (PI3K/Akt) pathways involved in the regulation of cell survival, cell cycle progression, cell growth and angiogenesis [23].

The endothelial cells secrete proteins to form new basement membranes, which are often immature and fail to reach final stages of maturation thus resulting in a leaky vasculature [6, 22, 23]. Endothelial cells communicate with the basement membrane and the ECM through integrin proteins (collagen, elastin, fibronectin and fibrillin) and proteoglycans for mechanical and physical support [25]. The basement membrane degrades to activate stroma thus allowing activated stroma to have a direct contact with tumour cells during tumorigenesis [26]. This induces alterations such as enhanced vascularity and increased ECM production, which are all essential for invasion.

During tumour metastasis, following cell detachment, tumour cells first undergo intravasation by first escaping the primary tumour site and enter the vasculature [6, 22, 23, 27]. Upon entering the vasculature, the tumour cells then adhere to endothelial cells during intravasation thereby changing the endothelial

barrier, which allows the tumour cells to migrate between two endothelial cells. This signifies that the interaction between the endothelial cells and tumour cells is reciprocal. The tumour cells can differentiate into endothelial cells within the tumour microenvironment to support and sustain tumour growth. Endothelial cells can also change cell fate and often undergo endothelial-mesenchymal transition (EMT), organised by TGF-β, EGF and bone morphogenetic protein (BMP), to cancer-associated fibroblasts (CAFs) during tumour progression [22, 24, 28]. This leads to a loss in cell-to-cell connection, detachment and elongation, enhanced migration, and a loss of endothelial properties. Cell adhesion establishes a tight a tight connection between cells as well as between cells and ECM thus activating cell proliferation and survival pathways [6]. Therefore, this loss in cell-to-cell connections enables cancer cells to transverse the vasculature (extravasation) and interact with pre-metastatic niches that permits cell proliferation and colonisation at the secondary sites [6, 24].

Furthermore, vascularization and endothelial cell expansion enhance tumour initiation and self-renewal properties of cancer stem cells within the tumour microenvironment. Endothelial cells secrete soluble factors that aid in the maintenance of stem cell properties in neural stem cells and activate cancer cells thereby promoting tumour growth [22]. Endothelial cells also secrete cytokines such as IL-8, which promote characteristics of cancer stem cells in glioblastoma including their migration and invasion abilities [22]. In a positive feedback loop of IL-8 mediated signalling, glioblastoma cells induce endothelial cell migration toward the tumour bulk thereby promoting brain tumour growth. In oesophageal cancer, epiregulin (EREG) overexpression is induced by endothelial cells and this leads to an increase in actin rearrangement, spheroid formation and enrichment of cancer stem cells [22].

4. Cancer-associated fibroblasts

CAFs are heterogenous populations of cells within the tumour microenvironment that have different phenotypic characteristics even within the same type of cancer. The origin of the cells is diverse; usually arise from tissue-resident fibroblasts but can also be derived from adipocytes, endothelial cells, pericytes, stellate cells and bone-marrow derived mesenchymal stem cells [6]. The role of these CAFs in the tumour microenvironment is to shape the tumour microenvironment via tumour proliferation, neoangiogenesis, invasion, metabolic reprogramming, extracellular matrix remodelling, immunosuppression, and metastasis [6, 22].

These cells facilitate the crosstalk between cancer cells and the tumour microenvironment. In the tumour microenvironment, a crosstalk between cancer cells and stromal cells leads to the secretion of factors such as TGF-β, PGDF, connective tissue growth factor (CTGF), hepatocyte growth factor (HGF) and fibroblast growth factor 2 (FGF2), which initiates the conversion of fibroblasts into cancer-associated fibroblasts (CAFs) [6, 22, 23, 29, 30]. Usually, their activation is via the NF-κB and JAK-STAT signalling pathways and is dependent on the secretion and release of signalling molecules such as TGF-β, stromal cell-derived factor-1 (CXCL12/SDF-1), platelet-derived growth factor α/β (PDGF α/β), basic fibroblast growth factor (b-FGF), RTK ligands, IL-1β and IL-6 by cancer or immune cells [22, 31]. The activation of CAFs is a common feature in tumorigenesis and these CAFs are perpetually activated unlike in normal tissues.

CAFs are a rich source of growth promoting molecules and proangiogenic factors as well as extracellular components such as growth factors, cytokines, and

extracellular matrix components. During cancer progression and metastasis, these cells secrete VEGF and TGF-β, which are crucial in angiogenesis and epithelial-mesenchymal transition (EMT) respectively [6, 22]. EMT is a vital step in metastasis via epigenetic changes, and it involves the loss of cell polarity and cell-to-cell adhesions by epithelial cells. In turn, these cells gain migratory and invasive phenotypes.

CAFs provide the physical scaffolding of cells and facilitate the migration of cancer cells through the tumour microenvironment by altering three-dimensional structure of ECM via the secretion of plasminogen activator protein and matrix metalloproteinase 3 (MMP3) that degrades E-cadherin to promote cancer cell invasion [8, 32, 33]. CAFs also migrate together with epithelial cancer cells thereby suggesting these cells play an important role in intravasation and extravasation of epithelial cells in metastasis by enhancing transmigration of cancer cells through endothelial cell layers [8, 32, 33]. In invasion of squamous cell carcinoma and breast cancer, CAFs create tracks through the ECM that cancer cells could not create on their own through Hippo-signalling dependent remodelling of ECM [8, 32, 33]. Activated fibroblasts also secrete elevated levels of ECM-degrading proteases such as matrix metalloproteases 2 and 9 (MMP2 and MMP9) [23]. Increase in ECM remodelling and degradation is associated with increase in metastasis.

Following activation by TGF-β, CAFs also modulate immune cells through factors such as monocyte chemoattractant protein-1 (MCP-1) and IL-1 leading to a pro-inflammatory microenvironment [23]. Activated CAFs also regulate collagen structure in the stroma of multiple solid tumours, including breast cancer. The cross-linking and alignment of collagen are associated with poor prognosis in cancer thus regulating invasion and metastasis [23]. CAFs also interact with the epithelium in breast cancer thus enhancing breast cancer progression and metastasis. In addition, CAFs also express programmed death ligand 1 (PD-L1) that leads to the suppression of $CD8^+$ T-cell immune responses and thence, progression of colon cancer [23].

5. Adipocytes

Adipocytes are specialised cells in the body that synthesise and store excess energy as fat thus regulating energy balance. Adipocytes are divided into lipid storing white adipocytes and thermogenic brown adipocytes [6, 23]. These cells secrete and release metabolites, enzymes, hormones, growth factors and cytokines through which they exert their effects on the tumour microenvironment. Adipocytes become activated when located near a growing tumour thus supplying pro-tumorigenic factors that will stimulate cancer cell invasion [6, 23]. These adipocytes are termed cancer-associated adipocytes (CAA).

In cancer progression and metastasis, these CAA form a crucial yet reciprocal relationship with the tumour cells. For example, in tumour microenvironment, adipocytes play very important role as the breast tissue is largely composed of white adipose tissue [6, 23]. This white adipose tissue enhances metastasis of breast cancer cells to the liver and lungs via paracrine signalling. Under the stimulation of breast cancer cells, adipocytes undergo lipolysis to breakdown lipid stores and make free fatty acids available for cellular uptake by cancer cells in response to local ECM remodelling [6, 23, 28, 32]. Cancer cells then use up these free fatty acids for respiration (energy production), formation of cell membrane, lipid bioactive molecules and/or package them into extracellular vesicles such as exosomes [6].

Adipose tissues play a vital role in the formation of mammary duct and vasculature by providing growth factors such as VEGF [23]. Therefore, adipocytes regulate angiogenesis and epithelium function. White adipocytes are also important in the production and secretion of hormones, especially leptin, oestrogen, and IGF-1 [6, 22, 23]. As a result, adipocytes directly promote tumour progression by releasing leptin that regulates food intake thus helping the body to maintain its weight. In breast cancer, leptin signalling enhances breast cancer cells by increasing receptor expression levels and activating different signalling pathways such as Notch, Wnt, HER2, AKT and NF-κB that have been implicated in tumorigenesis and tumour invasion [22]. Elevated levels of leptin in the BM microenvironment supports the proliferation and migration of cancer cells and protects them from cellular damage by suppressing caspase-3 activity [6, 23]. Most cancer patients are overweight making obesity a major risk factor for different types of cancer such as breast, pancreatic and ovarian. In addition, obesity-associated fatty acid binding protein (FABP4) is elevated in patients with breast cancer [22]. FABP4 increases tumour volume, tumour-initiating frequency and stemness markers via IL-6/STAT3/ALDH1 signalling pathway [6]. Breast cancer cells also interact with adipocytes via secretion of inflammatory factor IL-6 that plays a key role in maintaining cancer stemness. Adipocyte-secreted IL-6 also play important roles in Notch/Wnt/TGF-β signalling pathways by upregulating ALDH1A1 and LEF1 and AXIN2 gene expression in the Wnt pathways to promote invasion, angiogenesis, and metastasis of breast cancer [22]. Adipocytes also increases the metastasis of breast cancer cells via upregulation of PLOD2 expression. Elevated levels of IL-6 in the tumour microenvironment also regulates Bcl-xl and OCT4 expression in ovarian cancer through the regulation of STAT3, which contributes to chemoresistance [22]. Tumour-secreted soluble factors such as IL-6 and parathyroid hormone-related peptide (PHRP) stimulate browning (trans-differentiation of white to brown adipocytes) thereby resulting in an increase in energy expenditure of adipose tissues that contribute to cancer-associated cachexia [23]. Cancer-associated cachexia is a muscle wasting condition that negatively impacts patient quality life and as a result, is associated with poor prognosis.

Adipocytes also promote tumour progression indirectly, by activating macrophages. These tumour-associated macrophages (TAM) release growth factors, cytokines, inflammatory mediators, and proteolytic enzymes that mediate tumour growth, tumour cell migration and invasion [21]. Finally, adipocytes secrete metalloproteases such as MMP1, MMP7, MMP10, MMP11 and MMP14 that are important in modifying and degrading ECM [21, 34, 35]. These MMPs and serine proteases (such as urokinase plasminogen activator; uPA) are the major enzymes responsible for ECM degradation [29, 36, 37]). Both MMPs and serine proteases are involved in all stages of tumour progression such as angiogenesis, stroma invasion, intravasation, regulation of inflammation and metastasis [21, 29, 36, 37].

6. Extracellular vesicles and cancer metastasis

Extracellular vesicles (EVs) are nanoparticles released by different types of cells that contain a lipid bilayer structure [38, 39]. There are three major types of EVs: namely exosomes, apoptotic bodies and microvesicles [39, 40]. However, other types such as oncosomes, cytoplasts and exomeres have also been identified. These subtypes are characterised based on their sizes, biogenesis, origin (tumour-derived, stromal cell-derived etc), functions (immune-suppressing/stimulating-EVs, pro-apoptotic EVs etc)

and surface markers ($CD63^+$, $CD9^+$, $CD81^+$, or $EpCAM^+$ EVs) [38, 41]. Despite this heterogeneous population of EVs, each EV is unique thus the dynamic function of EVs is due to their highly heterogeneous characteristics, which makes it difficult to accurately differentiate these EV subtypes [42].

Apoptotic bodies are the largest EV in size and are produced by dying cells [38]. These EVs contain many intercellular materials such as intracellular fragments, cellular organelles, and cytosolic contents [38, 40]. Microvesicles are the second largest EV in size and originate from the outward budding or fusion of the cytoplasm membrane and are later released into the extracellular space [43]. They majorly contain lipids such as sphingolipids, cholesterol, and phosphatidylserine [40, 44]. Both apoptotic bodies and microvesicles are sometimes collectively called ectosomes and often originate via direct outward budding or blebbing of the plasma membrane [40]. Lastly, exosomes are bilayered membrane small extracellular vesicles of 40–200 nm size that are derived from the fusion of multivesicular bodies (MVB) into the plasma membrane and resulting release of intraluminal vesicles (ILVs) into the extracellular space through exocytosis [45, 46]. Therefore, any factor that may affect the plasma membrane may positively or negatively influence formation of these EVs.

These membrane-bound organelles function as important mediators of intercellular communication mechanism and often harbour bioactive molecules such as metabolites, proteins, RNA, DNA, and lipids that often reflect the parent cell [39, 47]. The lipid membrane of these EVs serves a protective shield for enclosed nucleic acids thereby protecting them from degradation by extravesicular nucleases [48]. Much of the RNA composition are from miRNAs, a class of non-coding RNAs that mediate post-transcriptional gene silencing in many biological processes [47, 49]. Once released, these vesicles are taken up by recipient cells and could influence the pathological and physiological functions in the recipient cells by activating different signalling pathways [49–52]. These EVs deliver genetic information to recipient cells, which affect signalling transduction pathways thereby regulating target gene expression and determining the function and fate of recipient cells such as apoptosis, growth, cell cycle, migration, and differentiation [49, 53, 54]. Internalisation of these vesicles into the recipient cells occur by endocytic process via phagocytosis, fusion with the cell membrane and interaction with receptors on the cell membrane [48, 55].

During tumorigenesis, the bidirectional cell-to-cell communication between tumour and healthy cells within the TME is one of the mechanisms that enable cancer progression and metastasis, and EVs mediate this intercellular communication [56]. EVs released by cancer cells are increasingly found circulating in body fluids such as blood, urine, saliva, ascitic fluid and milk whereby they enhance the proliferation and invasion of tumour cells in autocrine and paracrine manner [44, 55]. The hypoxic or metastatic status of the tumours plays an important role in sorting the loading of composition of EVs, which affects the functions of tumour-derived EVs in the TME [24, 56]. EVs shuttle regulatory molecules, including lipids, nucleic acids and proteins that induce the reprogramming and remodelling of the stroma by facilitating the development of a tumour-supportive environment [39, 47, 57, 58]. These tumour-derived EVs within the hypoxic microenvironment also drive Warburg effect thereby driving conversion of glucose mainly into lactate to meet energy requirements to ensure tumour survival [57, 58]. They also regulate the metabolism of lipids and amino acids by cancer cells to build biomass and provide more energy.

This leads to immunogenic stress thereby initiating immune changes within the TME and influencing cancer progression. Tumour-derived EVs inhibit immune response, promote the transformation of CAFs, and reprogram endothelial cells

function thus creating an anti-tumoral environment. Tumour-derived EVs interact with the host immune system and cause functional and phenotypic changes in immune cells such as natural killer (NK) cells, macrophages, T-cells, and B-cells thereby affecting the immune system homeostasis [57, 59]. EVs released by tumour cells also induce immunosuppressive or tumour-associated macrophages by NF-κB mediated metabolism and secretion of VEGF, IL-6, TNF-α and G-CSF thereby leading to cancer metastasis [60, 61]. Tumour-derived EVs also increase neutrophil mobilisation and activate regulatory T-cells that protects the tumour from $CD8^+$ T-cell mediated killing [59, 62, 63]. In addition, tumour-derived EVs activate or suppress NK cells depending on the type of tumour and express FasL and TRAIL on their membrane thereby directly influencing the apoptosis of $CD8^+$ cells [59, 62, 63]. However, tumour-derived EVs can also activate dendritic cells via delivery of tumour-derived antigens and stimulate a $CD8^+$-mediated anti-tumour response.

Tumour-derived EVs also regulate the pro-tumoral function of endothelial cells by sustaining the constant delivery of nutrients and oxygen from the vascular endothelium [58, 60, 64]. Under hypoxic conditions, tumour-derived EVs also promote the regulation of endothelial cell proliferation, migration, sprouting, branching, as well as tubular-like structure formation via delivery of miRNAs, mRNAs, and proteins hence tumour-derived EVs promote angiogenesis in different types of cancer, including hepatocellular carcinoma, colorectal cancer, cervical cancer, nasopharyngeal carcinoma, glioma, and lung cancer. Neoangiogenesis, secretion of growth factors and EVs, and inflammatory cells recruitment induce the formation of pre-metastatic niches, where new tumour cells extravasate, get arrested or colonise [43, 57, 58, 61]. This further ensures tumour metastasis. In addition to pre-metastatic niches, EVs are also involved in other processes of tumour metastasis such as EMT and organ-specific metastasis.

Under hypoxic conditions, tumour-derived EVs stimulate the transition of stromal cells into CAFs via TGF-β, which in turn increase shedding of EVs and induce ECM remodelling, angiogenesis, migration, and invasion of cancer cells via different signalling pathways [58, 60, 64]. Tumour-derived EVs enhance the ability of CAFs in response to metabolic environment by activating MYC signalling pathway in stromal cells resulting in rapid tumour growth. These EVs-bound factors modify the phenotype of cancer cells or tumour stromal cells to support the aggressive phenotype and tumour progression. CAFs regulate tumour microenvironment and transfer proteins, metabolites such as tricarboxylic acid (TCA) intermediates and lipids utilised by cancer cells via EVs to facilitate and promote tumour growth under nutrient deprivation conditions [14, 31, 59, 65]. CAFs-derived EVs also enhance EMT via release of factors such as fibronectin and vimentin that trigger the loss of tumour cell adhesion, as well as differentiation of osteoblasts and proliferation of osteoclasts, which regulate the microenvironment of bone metastasis.

7. Stromal cells and clinical therapy

Stromal cells have therapeutic potential in cancer treatment and targeting stromal components in combination with cancer cells may increase the efficacy of cancer therapy [4, 22]. Stromal signatures characteristic of different cancer subtypes may have clinical relevance and may even serve as a prognostic marker of the disease.

Previously, chemotherapeutic agents were used to target all cells within the tumour microenvironment however, efficacy of these therapies is reduced by the development of drug resistance [6, 59]. Drug resistance occurs primarily by activation or mutation of signal transducers downstream of the targeted molecule or secondarily when neoplastic cells originally sensitive to these drugs lose their response to drugs [7, 22, 59]. In recent years, advancement in therapeutic targeting of the tumour microenvironment has led to specific targeting of cells within the tumour microenvironment. Poorly vascularised stroma supports tumorigenesis and simultaneously forms a barrier for chemotherapeutic drugs making it as an attractive drug target [7, 22]. Since tumours require endothelial cells to form new blood vessels to help relieve oxygen deprivation and accumulate metabolic wastes, angiogenesis is one of the mechanisms targeted by chemotherapy.

Most of these drugs such as bevacizumab, aflibercept, sorafenib and ramucirumab target the VEGF-VEGF signalling pathway in diverse ways as this is associated with tumour progression and poor prognosis in breast cancer [6, 7, 51, 58]. Bevacizumab acts a neutralising antibody to VEFG that reduces vascular permeability thus affecting the first step of tumour stroma development however aflibercept acts a decoy receptor for VEGF. Sorafenib acts a tyrosine kinase inhibitor and ramucirumab acts as an antibody that blocks VEGF from binding to its receptor. However, these chemotherapeutic agents have shown limited success when administered to patients as a single agent. Most patients develop resistance or do not respond to this anti-angiogenic therapy. Metastatic tumour cells have a striking feature/ability to plastically adapt to different microenvironmental conditions and overcome a single-drug treatment [6, 7, 51, 58, 66].

To enhance success within the clinical settings, combination of these drugs or other drugs/approaches may likely prove to be beneficial. For example, combination of bevacizumab and PDL1 proved to be a success in the treatment of hepatocellular carcinoma and renal cancer [6]. Combination therapies targeting thyroid cancer cells and stroma may also offer treatment alternatives as there have been no convincing clinical studies that show the efficacy of tumour stroma inhibition in the most aggressive forms of thyroid cancer.

In addition, an antibody that blocks IL-8 has also been trialled to target the tumour-promoting effect of endothelial cells in glioblastoma with success [22, 23, 31]. This led to a marked reduction in tumour size. Other researchers have also shown that inhibition of IL-8 re-sensitised tumour cells to chemotherapeutic agents, cisplatin, and paclitaxel [22, 31]. Furthermore, CCL5 and IL-6 have also been shown to be associated with acquisition of chemoresistance [22, 31]. These suggest that these cytokines as well as other ligands of CXC chemokine receptors 1 and 2 could be very important in the induction of chemoresistance via recruitment of MSCs around the tumour. In addition, EGF secreted by endothelial cells has been associated with drug resistance in squamous cell carcinoma [22]. Nevertheless, there are very few existing FDA-approved treatments with limited efficacy, but new therapeutic targets and strategies will be identified as researchers continue to understand how the tumour microenvironment contributes to tumour progression and metastasis. There is potential for the use of chimeric antigen receptor natural killer cells, liver stellate cells and fibroblasts [6, 51].

In addition, CAFs may be novel and attractive targets for cancer therapy. CAFs also show the strongest expression level of the stem/mesenchymal transcription subtype of cancer. The crosstalk between CAFs and cancer stem cells is a convincing strategy for immune suppression, drug resistance, metastasis and stemness of

cancer cells [22, 23]. CAFs secrete TGF-β and HGF that contribute to drug resistance in tumour cells, including tamoxifen-associated resistance in breast cancer cells [22, 23]. As a result, some novel drugs target the interaction between CAFs and breast cancer cells as it is believed that CAFs increase interstitial pressure within the tumour thereby reducing the efficacy of drug delivery [22, 23, 66]. Also, pirfenidone, which is an anti-fibrotic agent with multiple functions including anti-TGF-β activity, was combined with doxorubicin to inhibit tumour growth and metastasis in a preclinical triple-negative breast cancer (TNBC) model [22, 23].

Targeting CAFs may affect other stromal cells such as polarising tumour-associated macrophages (TAM) and cause suppression of the cytotoxic activities of NK cells since CAFs are involved in promoting immunosuppression [23]. Partial depletion of stroma using CD40-activated macrophages has shown to improve patient survival and increase drug delivery into the tumour [22]. CAFs-induced EMT causes resistance to cisplatin in non-small-cell lung carcinoma [22, 28, 51]. Therefore, a build of CAFs in the tumour microenvironment is associated with poor prognosis in many cancers, including lung adenocarcinoma, squamous cell carcinoma and colorectal cancer, where it is associated with diseases reoccurrence [22, 23, 51]. However, these cells are associated with improved prognosis and overall survival in small lung cell carcinoma. Some researchers have illustrated that targeting Hedgehog-activated CAFs results in improved survival, chemosensitivity and reduced metastatic burden in breast cancer [22, 67].

However, depleting CAFs is not always beneficial and has been associated with increased angiogenesis and enhanced cancer cell properties in pancreatic cancer with shorter patient survival. Hence, these suggest that therapeutic targeting of these CAFs may ameliorate some cancers. Furthermore, the expression of CD44 on CAFs can be functional target for destroying cancer cells in the TME and TGF-β signalling mediated by CAFs plays a role in regulating cancer cells in gastric cancer. Inactivating CAFs or lowering the level of infiltrating CAFs in the TME are potential therapeutic strategies for reducing cancer stemness. Targeting myofibroblast-like CAFs using focal adhesion kinase (FAK) inhibitor resulted in a reduction of pancreatic cancer cells [22]. CAFs can also be targeted by inhibiting their activation by using drugs to target CAF-associated proteins such as fibroblast activation protein (FAP) and DNA methyltransferase 1 (DMNT1) [22]. Sibrotuzumab, a FAP-targeting antibody has been tested in the treatment of Phase II metastatic colorectal cancer whilst combination of DNMT1 and DNMT1 and Janus Kinase (JAK) signalling resulted in the normalisation of fibroblasts, but these failed to demonstrate efficacy [7, 22, 31]. Thus, it is noteworthy to mention that identifying and targeting fibroblasts is problematic due to heterogeneity of markers found on these cells. This, identifying CAFs aid define activate stroma borders and may even affect clinical response to treatment.

Furthermore, interaction between adipocytes and cancer cells has been therapeutically targeted using BMS309403, a FABP4-specific inhibitor in breast cancer [22]. The results revealed a reduction in tumour growth with changes in secretion of IL-6 and ALDH1 expression. Another drug, anti-leptin blocking peptide, impeded the migration of ovarian cancer cells thereby suggesting antibodies against leptin may be an effective therapy for different cancers, including breast cancer [22]. An agonist of Farnesoid X, GW4064, also decreases the signalling of leptin whilst doxorubicin and pirfenidone have been combined to reduce the progression and motility of tumours in the ECM components by inhibiting the production of collagen [22]. Decreased collagen production has also been induced by vaccination, which sensitises fibroblasts to CD8 T-cell attack thereby significantly increasing the uptake of chemotherapeutic drugs.

Furthermore, stromal cells also play an important role in regenerative therapy as well as haematopoietic stem cell transplantation (HSCT), which is the major treatment for cancer where they enhance HSC engraftment and prevent graft-versus-host disease (GVHD) [4, 7]. GVHD is a major complication of HSCT in the treatment of haematological malignancies. GVHD is caused by an attack on recipient tissues by transplanted immune cells.

8. Extracellular vesicles and clinical therapy

Since EVs reflect the physiological and pathological states of the parent cell, and control the energy production machinery of tumour cells, developing EVs as therapeutic strategy and drug delivery system is a promising clinical therapeutic strategy. In cancer, tumour-derived EVs have been identified in various types of body fluids of cancer patients and reflect the characteristics of the tumour cells [46, 62, 64]. Once internalised, alter the metabolism of recipient cells. Thus, EVs can act as biomarkers in disease prognosis, diagnosis, and treatment. Studies have shown the value of EV-derived proteins and miRNAs as prognostic and diagnostic markers in different types of cancer [44, 57, 64, 68].

Tumour-derived EVs have been shown to play vital roles in the resistance of tumour cells to anti-cancer therapy such as chemotherapy and radiotherapy [58, 62, 69]. This may be due to EVs' ability to mediate the transfer of miRNA, lncRNA and proteins associated with drug resistance to recipient cells. Proteins such as transient receptor potential channel 5 (TrpC5) and annexin-6 as well as miRNAs such as miR-310a and miR-17-92 family are highly expressed or upregulated in EVs released from patients with a poor response to chemotherapy and/or radiotherapy [40, 56, 57, 60]. Chemotherapy and radiation affect the function of EVs of target cells. Irradiated and drug-treated cells released EVs that confer a drug-resistant phenotype and reduce sensitivity of recipient cells to the chemotherapy/radiotherapy [57, 58].

However, EVs-derived biomolecules are also used as drug targets for cancer treatment. For instance, miRNAs found in EVs promote glycolysis of CAFs and are involved in pre-metastatic niche formation [53, 54, 64]. As a result, miRNA inhibitors have been used to target and reverse this effect. Fas ligand (FasL) found in EVs of activated T-cells also induce cancer metastasis upon interaction between cancer cells and FasL positive EVs [44, 54]. To ameliorate this effect, several studies have focused on using GW4869 to inhibit the secretion and release of EVs from cells with promising results [40, 54]. Thus, GW4869 might serve as a useful therapeutic strategy to inhibit communication different cells within the TME.

The cargo of EVs can also be useful as a drug delivery system in cancer treatment as EVs deliver bioactive molecules through the plasma membrane barriers with low cytotoxicity. In recent years, various molecules such as miRNAs, siRNAs and therapeutic molecules are incorporated into EVs to cross the blood-brain barrier to treat different types of tumours including brain tumours more efficiently [53, 64]. EVs have also been used to deliver chemotherapeutic drugs such as cisplatin and paclitaxel to increase concentration of these drugs in specific cells or organs [40, 53, 64]. Red blood cells-derived EVs have also been used to deliver drugs in liver cancer treatment through a macrophage-dependent manner [53, 54]. However, it is important to explore the process of cargo selection in the formation of EVs to focus the treatment strategy on specific molecules transported by EVs

from tumour cells or other cells within the TME. There are still discrepancies and difficulties surrounding the methods of isolation and purification of EVs from multiple body fluids [39, 42]. Thus, developing standard methods to isolate EVs may provide the gateway to further explore the possibility of targeting bioactive molecules in EVs and using EVs as a delivery system to carry therapeutic drugs to cells within the TME for cancer treatment.

9. Conclusion

Cancer metastasis is the leading cause of cancer morbidity and death. Stromal cells such as endothelial cells, cancer-associated fibroblasts and adipocytes are all involved in cancer development, progression, and metastasis by aiding the spread of cancer from the point of origin to a distant disconnected part of the body. In recent years, clinicians have focused on these stromal cells to provide clinical therapy to patients with cancer. However, this field is relatively new and further research into the roles of these cells in cancer metastasis and the molecular mechanisms should be explored. This will provide a molecular understanding of different types of cancer, and lead to the development of different therapies that will enhance patient survival.

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