Chapter 13
Intercellular Communication, the Tumor Microenvironment, and Tumor Progression

Mei-Fong Pang and Celeste M. Nelson

Abstract Within a solid tumor, cancer cells interact with normal host cells as well as with insoluble and soluble factors, including extracellular matrix proteins and chemokines. Here, we survey the molecular mechanisms by which cancer cells interact with their surrounding microenvironment, with a particular focus on intercellular communication. This host-tumor crosstalk provides key signals that direct the growth, migration, and dissemination of cancer cells. Approaches that disrupt or coopt intercellular communication between the tumor and its host are exciting and potentially powerful anti-cancer therapeutic strategies.

Keywords Stroma • Microenvironment • Metastasis

Abbreviations-activated

CAF	Cancer-associated fibroblast
CAR	Coxsackie-adenovirus receptor
CC	Chemokine
CCR	Chemokine receptor
CSC	Cancerstem cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
GJ	Gap junction
GJIC	Gap junction intercellular communication
HCC	Hepatocellular carcinoma
HGF	Hepatocyte growth factor
ICAM	Intercellular adhesion molecule

M.-F. Pang • C.M. Nelson (✉)
Departments of Chemical and Biological Engineering, Princeton University,
Princeton, NJ 08544, USA

Departments of Molecular Biology, Princeton University,
303 Hoyt Laboratory, William Street, Princeton, NJ 08544, USA
e-mail: celesten@princeton.edu

M. Kandouz (ed.), *Intercellular Communication in Cancer*,
DOI 10.1007/978-94-017-7380-5_13

IL	Interleukin
LN	Lymph node
MSC	Mesenchymal stem cell
MVB	Multi-vesicular body
PDGF	Platelet-derived growth factor
SLN	Sentinel lymph node
TAK	Transforming growth factorkinase
siRNA	Short interfering RNA
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor

13.1 Introduction

13.1.1 The Tumor Microenvironment

Interactions between cells and their surrounding microenvironment are crucial to maintain functional tissue homeostasis. Defects in these interactions result in tissue pathology and disease progression. Solid tumors consist of cancer cells and the surrounding tumor microenvironment. The cellular components of the tumor microenvironment include fibroblasts, endothelial cells, and inflammatory cells. The physical and chemical components include the extracellular matrix (ECM) and diffusible molecules such as dissolved oxygen, cytokines, and growth factors [1–3] (Fig. 13.1). Cancer cells interact dynamically and co-evolve with this complex surrounding microenvironment [4]. Crosstalk between cancer cells and the tumor microenvironment can affect the behavior of cancer cells and promote tumor progression by conferring cancer cells with the ability to migrate, invade, and metastasize [1, 3].

13.2 Acquisition of Invasive and Metastatic Properties during Tumor Progression within the Tumor Microenvironment through Intercellular Communication

Several types of specialized intercellular junctional proteins maintain epithelial cell contacts with neighboring cells, including tight junctions, adherens junctions, and gap junctions [5]. Tight junctions are comprised of transmembrane proteins such as coxsackie adenovirus receptor (CAR) and occludin, which seal the space between

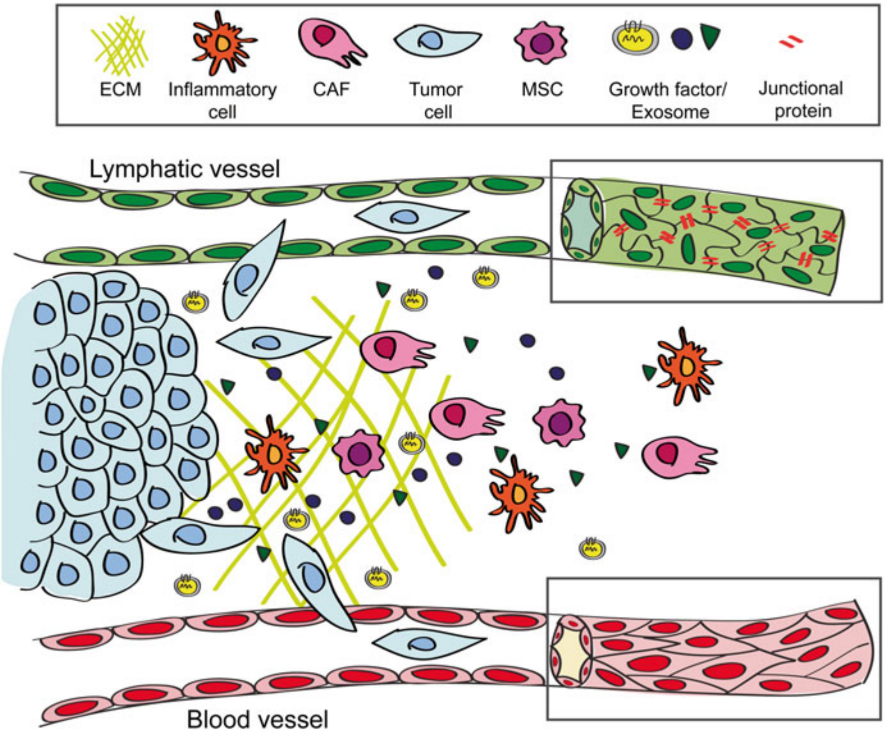

Fig. 13.1 Thetumor microenvironment. Intercellular interactions within the tumor microenvironment drive tumor progression to an invasive state. The physical components of the tumor microenvironment, including the ECM, can influence the metastatic properties of cancer cells. Intercellular communication between tumor cells and other cells including vascular endothelial cells, CAFs, and inflammatory cells directly or indirectly permits the acquisition of an invasive and migratory phenotype. For example, elevated levels of TGFβ secreted by stromal cells within the tumor microenvironment can induce EMT and CSC properties in cancer cells, leading to tumor dissemination and metastatic spread

neighboring cells and prevent the passage of molecules and ions. Adherens junctions form from complexes of E-cadherin that anchor cells to each other. Gap junctions (GJs) are communicating junctions made up of connexins that allow direct intercellular communication between neighboring cells [6]. Intercellular communication is important for the maintenance of homeostasis as well as tumor progression. Cancer cells use these various junctional complexes to transmit signals to their neighbors and respond collectively to different conditions [7]. Cancer cells can communicate with each other directly through these junctions [8, 9] or indirectly through paracrine signaling via the secretion of growth factors [10], chemokines, [11] and exosomes [12].

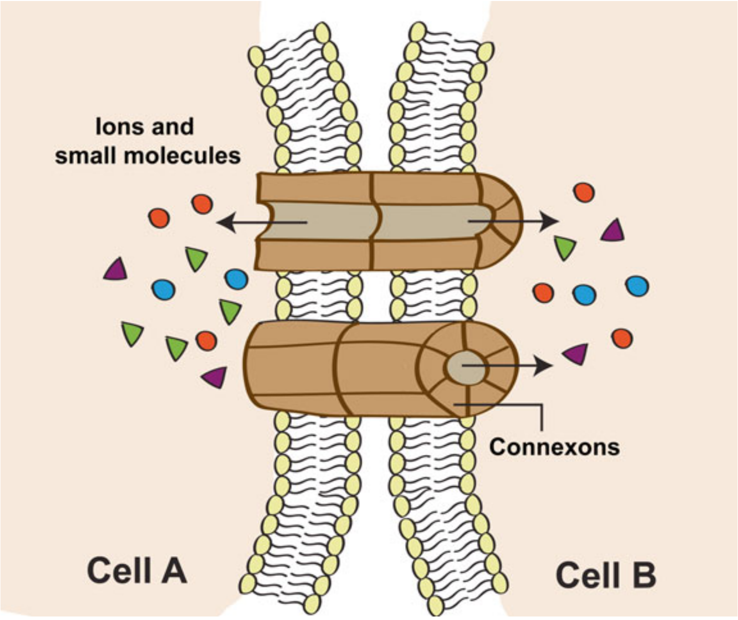

Fig. 13.2 Gap junctions. GJs are composed of connexons that each assemble from six subunits of GJ proteins, connexins. GJs connect the cytoplasm of adjacent cells and allow direct communication between cells through the passage of ions and small molecules

13.2.1 Direct Intercellular Communication Via Gap Junctions

GJs are gated intercellular channels composed of connexons that each assemble from six connexins (Fig. 13.2). The opening of GJ channels allows the cell-to-cell transfer of small molecules (M_r <1000) including ions [13], short interfering RNAs (siRNAs) [14, 15], and metabolites [16]. Gap junction intercellular communication (GJIC) thus allows for the direct communication between the cytoplasms of adjacent cells. GJIC can occur between the same type of cells (homospecific) or between different types of cells (heterospecific) [17, 18]. GJIC plays a critical role in maintaining cellular homeostasis by regulating processes including proliferation, differentiation, and barrier function [19–21]. Loss of GJs and altered localization of connexins are often observed in human cancers [9, 22–25]. Intercellular communication can be measured by passing the current from a microelectrode from one cell to an adjacent cell [26]. Using this method, Loewenstein and Kanno found that GJIC was reduced between cancer cells [26]. GJIC can also be measured using a dye-coupling assay in which fluorescent tracers are microinjected into individual cells and the transfer of dye into the adjacent cells is monitored and quantified [22]. Dye-coupling assays have revealed an absence of GJIC between cancer cells and the adjacent normal cellular compartment in primary human liver tumors. In these cells, connexin 32 (Cx32) localizes to the cytoplasm instead of at the plasma membrane

[22]. Similarly, immunoelectron microscopy analysis has revealed that squamous carcinoma cells have poorly developed GJs, with some of the connexins accumulating in the cytoplasm [27]. These studies suggest that aberrant localization of connexins might be one mechanism by which cancer cells inactivate GJIC. Conversely, exogenous expression of Cx32 into communication-deficient cancer cells can restore GJIC and attenuate tumor growth in vivo [9]. Consistent with these findings, Cx32-deficient mice are significantly more susceptible to developing hepatic tumors both spontaneously as well as when induced by treatment with diethylnitrosamine [28]. Similarly, Cx43-heterozygous mice are more prone to developing lung tumors in response to urethane administration than are wild-type mice [29]. These findings suggest a role for GJIC in tumorigenesis.

Furthermore, disrupting GJIC can alter metastatic potential. Loss of GJs is associated with enhanced migration capacity of cancer cells. Introducing the metastasis suppressor gene, breast cancer metastasis suppressor-1 (BRMS1), into highly metastatic breast cancer cells can restore GJIC to a level similar to that of normal breast tissue and reduce metastatic potential [30]. Similarly, ectopic expression of Cx32 increases intercellular adhesion between HeLa cells and suppresses their ability to migrate and invade [31]. Treatment with the GJIC inhibitor, oleamide, reverses the effect of Cx32 overexpression, suggesting that GJIC itself regulates the migratory and invasive phenotype of tumor cells [31]. The expression of Cx43 is downregulated in breast cancer cells compared to normal breast epithelial cells [30, 32]. Overexpression of Cx43 reduces breast cancer cell invasiveness by suppressing the expression of N-cadherin [33] and preventing tumor cell adhesion to endothelial cells [33]. Consistently, injection of Cx43-expressing breast cancer cells into immunocompromised mice results in smaller tumors and fewer lung metastases compared to control cells [34], suggesting that Cx43 alters the metastatic potential of breast cancer cells by regulating N-cadherin. Cx43-knockout mice have disrupted cell polarity and display defects in epithelial-mesenchymal transition (EMT) in the epicardium [35].

Heterospecific GJIC between tumor cells and other cell types within the tumor microenvironment may affect the metastatic potential of cancer cells [30]. It has been shown that the growth of transformed cells is inhibited where there is strong heterologous communication with the adjacent non-transformed cells. When the heterologous communication is blocked chemically, the growth inhibitory effect is abolished, suggesting an active role for GJIC in transmitting signals for growth control [36]. Electron microscopy analysis has revealed that leukocytes induce the expression of Cx43 and formation of GJs between themselves and endothelial cells during inflammation [37], suggesting that communication via GJIC may mediate leukocyte extravasation. Similarly, studies indicate that cancer cells and endothelial cells interact with each other to mediate cancer cell extravasation to distant sites [38–40]. Ectopic expression of Cx43 in breast cancer cells that lack GJIC promotes the establishment of functional heterologous communication with vascular endothelial cells and enhances transendothelial migration of the cancer cells [39]. Functional GJs are formed at the contact sites between cancer cells and endothelial cells where metabolic coupling occurs [40]. The modulation of GJIC between cancer cells and endothelial cells depends on the ability of cancer cells to adhere to the endothelium [40]. Breast cancer cells that overexpress Cx43 have an enhanced capacity to adhere to the pulmonary endothelium, whereas breast cancer cells overexpressing a dominant-negative mutant of Cx43 fail to adhere [41]. Elevated levels of Cx43 have also been found at contact sites between cancer cells and endothelial cells [40, 41].

Dye transfer assays have revealed that ectopic expression of Cx26 can induce the formation of heterologous GJs between melanoma cells and vascular endothelial cells in a coculture system [42]. The Cx26-expressing melanoma cells were also found to be more metastatic than mutant Cx26-expressing cells [42]. Consistent with these studies, Cx26 has been implicated in the metastasis of human cancers [43–46]. Immunohistochemical analysis shows a strong correlation between Cx26 expression and lymphatic vessel invasion [46]. Cx26 might potentiate lymphatic vessel invasion through the formation of heterologous GJIC between the cancer cells and lymphatics [46]. These data indicate that formation of heterologous GJIC between cancer cells and endothelial cells may play a role in cancer cell extravasation. Recently, bone marrow stromal cells were shown to transfer CXCL12-specific microRNAs (miRNAs) to breast cancer cells through GJIC [47]. Since reducing the levels of CXCL12 inhibits the proliferation of breast cancer cells and induces quiescence, this GJIC-mediated transmission of miRNA might contribute to breast cancer cell dormancy [47]. Direct intercellular communication involving GJs between cancer cells and other cell types within the tumor microenvironment can promote tumor progression, so therapeutic targeting of GJIC may inhibit the metastatic spread of cancers.

13.2.2 Indirect Intercellular Communication: Paracrine Signaling via Exosomes, Growth Factors, and Cytokines

Although cell-cell communication can occur in a contact-dependent manner via GJIC, cells can also communicate with distant target cells through the secretion of signaling molecules such as growth factors, cytokines, and exosomes [48]. The secreted molecules act as local mediators to regulate cell signaling within the surrounding microenvironment, in a process known as paracrine signaling. Exosomes are cup-shaped vesicles ranging from 50-100 nm in diameter [49]. These naturally occurring nanoparticles are generated from multivesicular bodies (MVBs). Fusion of MVBs with the plasma membrane leads to release of exosomes via exocytosis into the surrounding tumor microenvironment and circulation [49, 50–52] (Fig. 13.3). Accumulating evidence suggests that exosomes are important mediators of intercellular communication. Exosomes contain cargos of bioactive molecules including DNA fragments [53], mRNAs [54, 55], miRNAs [54–56], and proteins [57, 55], which can be transferred into recipient cells. Cancer cells [58–61] and cancer-associated fibroblasts (CAFs) [62] have been found to release exosomes that

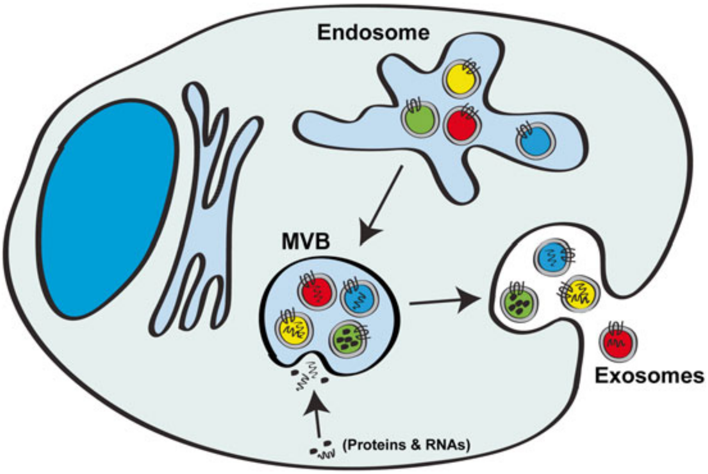

Fig. 13.3 Mechanisms ofexosomebiogenesis. Exosomes are derived from late endosomes called MVBs. Small molecules such as proteins and RNA are sorted in MVBs, and their fusion with the plasma membrane leads to secretion of exosomes via exocytosis

communicate with distant cell types to modulate immune evasion [63], angiogenesis [58], drug resistance [64], and formation of the pre-metastatic niche [60]. Higher levels of circulating exosomes are found in cancer patients than in healthy individuals [65, 66]. Cancer patients with high levels of exosomes have poorly differentiated tumors and a reduced rate of survival [67]. In vivo imaging of tumor-derived exosomes using GFP-labeled CD63, an exosome marker, in an orthotopic mouse model has revealed that breast cancer cells release exosomes into the tumor microenvironment and circulation. These tumor-derived exosomes are taken up by other cancer cells and CAFs in mice with breast cancer metastases, indicating that tumor-derived exosomes might be involved in promoting metastasis [52]. Membrane fusion assays and confocal microscopy analysis have revealed that the uptake of exosomes is mediated through direct fusion of the exosome membrane with the plasma membrane [68].

Double-stranded DNA (dsDNA) has been found in exosomes [69, 70] and whole genome sequencing using genomic DNA isolated from exosomes of cancer cell lines and serum from cancer patients has detected mutations in oncogenes and tumor suppressor genes including KRAS and p53 [69]. These findings suggest the possibility of using exosomes as a circulating biomarker to detect cancer. Microarray analysis of exosomes derived from mouse and human mast cell lines has revealed the presence of mRNAs and small RNAs such as miRNAs [54]. In vitro translation assays have demonstrated that these exosomal mRNAs are functional and can be translated into proteins [54]. Bioinformatics analysis using miRNA profiling of hepatocellular carcinoma (HCC) has identified a set of miRNAs that control the regulation of transforming growth factor-activated kinase-1 (TAK1) [56], which plays a crucial role in liver cancer [71]. Incubating cells with exosomes derived from HCC constitutively activates TAK1 and TAK1-associated signaling pathways [71]. HCC-derived exosomes can also regulate anchorage-independent growth in recipient cells, suggesting that the transfer of exosomal-derived miRNA can affect tumorigenesis [71].

Exosomes derived from highly metastatic melanomas can drive bone marrow progenitor cells towards a pro-metastatic phenotype through the regulation of the receptor tyrosine kinase, Met [60]. Inhibition of exosome production and down-regulation of Met expression in exosomes abolishes the pro-metastatic phenotype of bone marrow progenitor cells and suppresses primary tumor growth and metastasis in a xenograft mouse model [60]. Tumor-derived miRNA from metastatic adenocarcinoma can induce the formation of the pre-metastatic niche to support the growth of poorly metastatic adenocarcinoma [72]. Sentinel lymph nodes (SLNs) enriched with melanoma-derived exosomes promote the recruitment of melanoma cells [73]. In addition, exosomes isolated from melanoma can enhance the trafficking of melanoma cells towards lymphatic vessels in vivo by preconditioning the SLNs for tumor growth and tumor angiogenesis [73].

Bone marrow stromal cells secrete exosomes containing miRNA against CXCL12 to induce a quiescent state in breast cancer cells, indicating a role for stroma-derived exosomes in regulating breast cancer dormancy [47]. Evading immune destruction is one of the hallmarks of cancer [74] and cancer cells can communicate with immune cells to evade immune surveillance [63]. For example, exosomes derived from human prostate cancer cells induce the apoptosis of T cells in a Fas ligand (FasL)-dependent manner [63]. Blocking the Fas-FasL interaction using a FasL-neutralizing antibody prevents exosome-induced T cell apoptosis [63]. Exosomes secreted by chemoresistant breast cancer cells, which contain miRNAs that modulate the cell cycle and apoptosis, can also confer drug resistance to chemosensitive breast cancer cells [64]. For example, HER2-positive cancer cells release exosomes that can alter chemosensitivity to trastuzumab, a drug that targets the HER2 receptor [75]. HER2-positive exosomes abolish the antitumor activity of trastuzumab [75].

In addition to releasing exosomes to drive tumor progression, cancer cells and the surrounding stroma also communicate with each other through the secretion of various inflammatory cytokines, growth factors, and proteases to shape a metastasis-permissive tumor microenvironment [76]. Inflammatory cytokines and growth factors such as transforming growth factor-beta (TGFβ) [77], tumor necrosis factor-alpha (TNFα), epidermal growth factor (EGF) [78], hepatocyte growth factor (HGF) [79] and interleukins (IL) including IL-6 [80] and IL-8 [81] all induce EMT, a process in which polarized epithelial cells become highly migratory and invasive mesenchymal-like cells [82]. The activation of EMT inducing-transcription factors such as Snail, Slug, Twist, and Zeb alters gene expression, apical-basal polarity, and reduces cell-cell interactions [83, 84]. EMT is essential for tissue and organ generation during embryogenesis [85], and has also been found to be reactivated during pathological conditions such as fibrosis, wound healing, and cancer [86]. Although still somewhat controversial [87], accumulating evidence suggests that EMT facilitates tumor cell dissemination and metastasis during tumor progression [86]. The induction of EMT in cancer cells results in the acquisition of a metastatic phenotype [88]. Furthermore, recent studies suggest that the induction of EMT endows cells with cancer stem cell (CSC) properties [89]. These findings suggest the possibility of disrupting paracrine-mediated intercellular communication to inhibit tumor progression.

13.3 Tumor Cell Dissemination

13.3.1 Organ-Specific Metastasis: The Role of Chemokines

Over a century ago, Stephen Paget examined the autopsy reports of 735 breast cancer cases and found that the distribution of metastasis was not random [90]. Paget suggested that tumor cells ("seed") can only grow and survive in appropriate microenvironments ("soil") [90]. Various types of cancers have specific patterns of metastasis [91] and it is now understood that the specificity of metastasis is mediated in part by specific molecules expressed by cancer cells and the resident host cells at metastatic sites. In particular, chemokines (CCs) and chemokine receptors (CCRs) mediate the circulation and homing of immune cells during inflammation and the immune response [92] (Fig. 13.4). CCs are small, secreted peptides that mediate the chemotactic migration of cells expressing appropriate CCRs [92], which are G protein-coupled receptors. For example, CCL21/CCR7 and CXCL12/CXCR4 are involved in the homing of dendritic cells to lymph nodes during inflammation [93]. Binding of CCs to CCRs activates signaling pathways that regulate cell polarity, migration, and adhesion [92]. Different cancer cells express different CCRs, suggesting that cells may respond specifically to chemotactic forces generated by CCs secreted by target destinations [94]. Therefore, cancer cells may exploit CC/CCR signaling during tumor dissemination [95]. In addition to mediating directional migration, CCs also stimulate the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [96].

13.3.2 Role of Chemokines in Hematogenous Dissemination: Tumor-Vascular Endothelial Interactions

Metastasis is a complex process that involves a defined series of events. Cancer cells must detach from the primary tumor site; intravasate into the blood or lymphatic vasculature; survive through the circulation; extravasate from the vasculature and colonize the distant site [97]. Intercellular communication between tumor cells and the stromal compartment plays an important role in facilitating hematogenous dissemination [98, 99]. Coculture of tumor cells with platelets and leukocytes can activate microvascular endothelial cells [100]. Activated microvascular endothelial cells produce elevated levels of the inflammatory CC, CCL5, to recruit inflammatory monocytes to shape a pro-metastatic microenvironment [100]. Administration

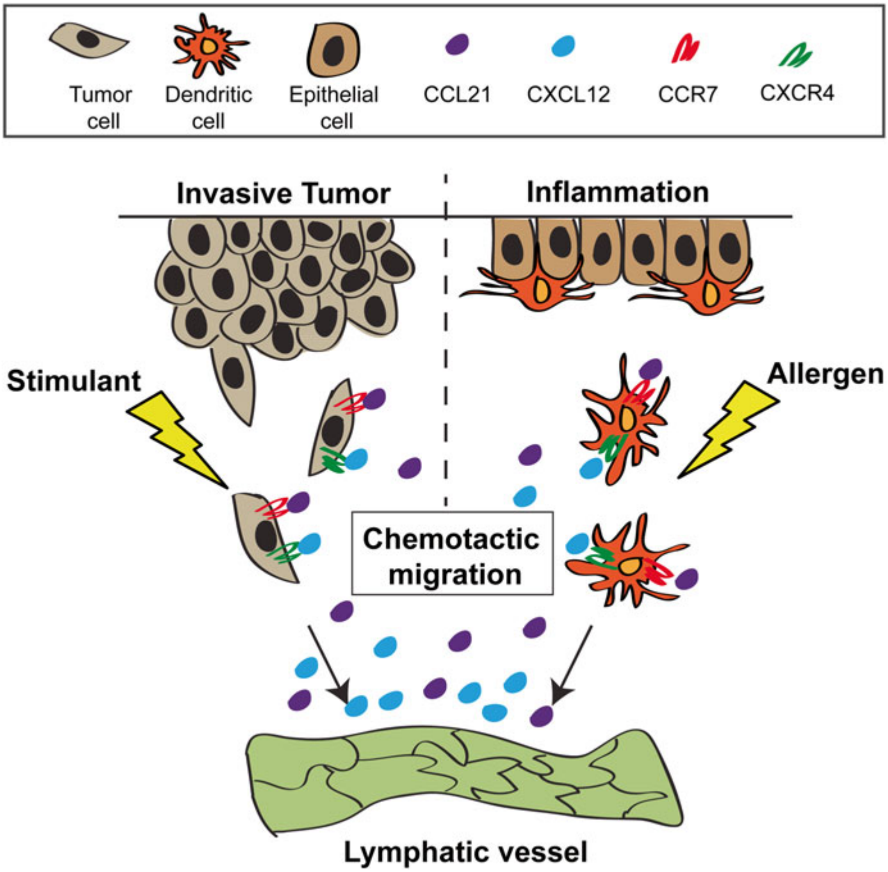

Fig. 13.4 CC and CCR expression during lymphatic homing. During inflammation, DCs are activated by allergens. Activated DCs upregulate the lymphatic homing receptors such as CCR7 and CXCR4. Induction of CCR7 and CXCR4 mediates the chemotactic migration of activated DCs towards the lymphatic vessels, which secrete specific ligands, CCL21 and CXCL12. Overexpression of CCR7 and CXCR4 has been implicated in LN metastasis in various human cancers. Therefore, it is thought that tumor cells hijack the DC route to metastasize towards the lymphatics

of CCL5 antagonist abolishes CCL5-dependent monocyte recruitment and strongly suppresses tumor metastasis in an experimental metastasis model, suggesting a pro-metastatic role for CCL5 [100]. Furthermore, components of the stromal compartment, such as mesenchymal stem cells (MSCs), can affect the phenotype of tumor cells through the secretion of CCL5. Binding of CCL5 to CCR5-expressing tumor cells significantly enhances metastasis and promotes tumor cell dissemination [101]. Microarray analysis of human breast cancer specimens shows an increase in the expression of CCL5 and its receptor, CCR5. Consistently, inhibiting CCR5 reduces breast cancer invasiveness in culture and decreases metastatic colonization in vivo [102].

CCs can also regulate physiological and pathological angiogenesis [103]. CXCL1, CXCL2, CXCL8 and CCL2 are among the pro-angiogenic CCs [104, 105]. CCL2 can induce the expression of vascular endothelial growth factor-A (VEGF-A) in prostate cancer cells, which suggests that CCL2 can promote tumor-induced angiogenesis indirectly [106]. Xenograft studies using CXCL8-expressing melanoma cells revealed that tumor growth, angiogenesis, and metastasis are significantly reduced in CXCR2-knockout mice, indicating the importance of CXCL8/CXCR2 signaling in regulating tumor-associated angiogenesis and dissemination [107].

13.3.3 Role of Chemokines in Lymphatic Dissemination: Tumor-Lymphatic Endothelial Interactions

Histopathological analysis of human biopsies has often revealed the presence of tumor cells in close proximity to peritumoral lymphatic vessels [108]. Malignant cancer cells can induce tumor lymphangiogenesis through the secretion of lymphangiogenic factors including VEGF-A, VEGF-C, VEGF-D, and platelet-derived growth factor (PDGF)-BB for tumor dissemination [109, 110]. In addition to tumor lymphangiogenesis, CC-mediated interactions between cancer cells and lymphatic endothelial cells can also enhance cancer cell dissemination towards lymphatic vessels [109]. CC gradients generated by lymphatic vessels, including CCL21 and CXCL12, act as guidance cues to mediate the directional migration of cancer cells [111]. Lymphatic endothelial cells have unique, semi-open, and discontinuous ‘button-like’ junctions within the lymphatic vessels [112]. Tumor cells might have more access to lymphatic vessels due to this ‘leaky’ structure, and may use a similar mechanism of entry into the lymphatics as dendritic cells (DCs).

Several CC/CCR pairs, including CCL21/CCR7 and CXCL12/CXCR4, have been implicated in lymph node (LN) metastasis in various cancers [113–115]. Lymphatic vessels and secondary lymphoid organs secrete high levels of CCL21, which is the ligand for its cognate receptor, CCR7 [111, 116]. CCR7 is indispensable for lymphatic homing, as mature DCs in CCR7-deficient mice fail to migrate towards the draining lymph nodes [93]. Under normal conditions, DCs increase their expression of CCR7 upon activation and during maturation. Binding of lymphatic-secreted CCL21 to CCR7 mediates directional migration of DCs towards the lymphatics [111]. Recently, Shields and co-workers showed that metastatic tumor cells express CCR7 and secrete CCR7 ligands autologously [117]. Under the influence of autologous chemotaxis and interstitial flow caused by draining lymphatics, these tumor cells were guided to the lymphatics [117]. Ectopic expression of CCR7 in B16 melanoma cells results in a significant increase in the number of LN metastases in a xenograft mouse model [118]. LN metastasis in CCR7-expressing B16 melanoma mice is inhibited after treatment with anti-CCL21-blocking antibodies, indicating a role for the CCL21/CCR7 axis in lymphatic dissemination of cancer cells [118].

In addition to CCL21/CCR7, elevated levels of CXCL12/CXCR4 have been associated with LN metastasis [119–121]. CXCL12 is expressed by the high endothelial venules (HEV) and the T cell zones of the spleen and LNs [122]. Similar to CCL21, CXCL12 plays a crucial role in recruiting immune cells during the initiation of the immune response [123] (Fig. 13.4). T cells, B cells, and mature DCs express the receptor for CXCL12, CXCR4 [124, 125]. Activation of CXCR4 by CXCL12 increases the adhesion of tumor cells to endothelial cells by upregulating the expression of β1-integrin [126]. Tumor-associated lymphatic vessels can mediate targeted migration of CXCR4(+)/CD133(+) melanoma cells through the secretion of CXCL12 [127]. Inhibiting CXCR4 signaling using the specific inhibitor, AMD3100, reduces axillary LN metastasis, indicating a role for CXCL12/CXCR4 in LN metastasis [127]. Since both CCR7 and CXCR4 are lymphatic-homing receptors for DCs and have been closely linked with high incidence of LN metastasis [115, 128], it is plausible that metastatic tumor cells might exploit the DC trafficking route during inflammation by manipulating their chemotactic interaction with the lymphatics via CCs/CCRs to mediate lymphogenic dissemination.

13.4 Therapeutic Interventions Targeting Intercellular Communication

Increasing GJIC in cancers is beneficial to anti-cancer therapy [129]. Treatments that combine a GJ enhancer, quinolones (PQs), with cisplatin decrease mammary tumor growth compared to cisplatin treatment alone in a mouse xenograft model of breast cancer [130]. Histology analysis revealed that PQ treatment upregulates the expression of the GJ proteins, Cx43 and Cx26, in the tumor. Similarly, targeting paracrine signaling appears beneficial. The GE11 peptide can bind to EGF receptor with high specificity [131]. Engineered exosomes that contain GE11 allow for targeted delivery of let-7a miRNA to EGFR-positive breast cancer cells in a xenograft mouse model [132]. Systemic administration of GE11-positive exosomes loaded with let-7a miRNA suppressed tumor growth in vivo, suggesting the usefulness of exosomes to deliver miRNA as a therapeutic approach [132]. Engineered exosomes may also be used for targeted delivery of the chemotherapeutic drug, doxorubicin (Dox), to the tumor in a mouse model. Dox was encapsulated into exosomes engineered to express αv-integrin-specific iRGD peptide on the surface. Dox-containing iRGD exosomes were able to bind to αv-integrin-positive tumor cells and suppress tumor growth in tumor-bearing mice more efficiently than non-targeting control exosomes [133].

Chemotherapy-resistant breast cancer cells express high levels of TGFβ and acquire CSC-like properties [134]. The chemotherapeutic drug, paclitaxel, enriches the population of breast CSCs by upregulating TGFβ and IL-8 signaling. Co-administration of paclitaxel and the TGFβ type I receptor kinase inhibitor, LY2157299, into a xenograft mouse model inhibited tumor reestablishment, suggesting that this combination treatment blocks the development of chemoresistance and CSC expansion in breast cancer [135]. Another study showed that a combination treatment of CXCR4-specific inhibitor, AMD3100, and the chemotherapy drug, docetaxel, enhanced the antitumor effect of docetaxel in a tumor-bearing mouse model. These data reveal the possibility of using the CXCR4 inhibitor as a chemosensitizer in cancer treatment [136]. It is clear from these studies that targeting intercellular communication can attenuate tumor growth and metastatic spread. However, more studies need to be carried out to assess the usefulness of therapeutic interventions that target intercellular communication within the tumor microenvironment.

13.5 Conclusion

It is well established that intercellular communication between cancer cells and other cells within the surrounding tumor microenvironment is critical for tumorigenesis and tumor progression. Therefore, understanding the mechanisms underlying these interactions might lead to the development of novel therapeutics to block tumor progression and metastasis.

References

1. Mueller MM, Fusenig NE (2004) Friends or foes – bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 4(11):839–849
2. Korkaya H, Liu S, Wicha MS (2011) Breast cancer stem cells, cytokine networks, and the tumor microenvironment. J Clin Invest 121(10):3804–3809
3. Calorini L, Bianchini F (2010) Environmental control of invasiveness and metastatic dissemination of tumor cells: the role of tumor cell-host cell interactions. Cell Commun Signal 8:24
4. Polyak K, Haviv I, Campbell IG (2009) Co-evolution of tumor cells and their microenvironment. Trends Genet 25(1):30–38
5. Matter K, Aijaz S, Tsapara A, Balda MS (2005) Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. Curr Opin Cell Biol 17(5):453–458
6. Evans WH, Martin PE (2002) Gap junctions: structure and function (review). Mol Membr Biol 19(2):121–136
7. Stains JP, Civitelli R (2005) Gap junctions regulate extracellular signal-regulated kinase signaling to affect gene transcription. Mol Biol Cell 16(1):64–72
8. Yamasaki H (1990) Gap junctional intercellular communication and carcinogenesis. Carcinogenesis 11(7):1051–1058
9. Eghbali B, Kessler JA, Reid LM, Roy C, Spray DC (1991) Involvement of gap junctions in tumorigenesis: transfection of tumor cells with connexin 32 cdna retards growth in vivo. Proc Natl Acad Sci U S A 88(23):10701–10705
10. Imagawa W, Pedchenko VK, Helber J, Zhang H (2002) Hormone/growth factor interactions mediating epithelial/stromal communication in mammary gland development and carcinogenesis. J Steroid Biochem Mol Biol 80(2):213–230

11. O'Hayre M, Salanga CL, Handel TM, Allen SJ (2008) Chemokines and cancer: migration, intracellular signalling and intercellular communication in the microenvironment. Biochem J 409(3):635–649
12. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L (2010) Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 78(9):838–848
13. Qu Y, Dahl G (2002) Function of the voltage gate of gap junction channels: selective exclusion of molecules. Proc Natl Acad Sci U S A 99(2):697–702
14. Wolvetang EJ, Pera MF, Zuckerman KS (2007) Gap junction mediated transport of shRNA between human embryonic stem cells. Biochem Biophys Res Commun 363(3):610–615
15. Valiunas V, Polosina YY, Miller H, Potapova IA, Valiuniene L, Doronin S, Mathias RT, Robinson RB, Rosen MR, Cohen IS, Brink PR (2005) Connexin-specific cell-to-cell transfer of short interfering rna by gap junctions. J Physiol 568(Pt 2):459–468
16. Chang Q, Tang W, Ahmad S, Zhou B, Lin X (2008) Gap junction mediated intercellular metabolite transfer in the cochlea is compromised in connexin30 null mice. PLoS One 3(12), e4088
17. Yamasaki H, Mesnil M, Omori Y, Mironov N, Krutovskikh V (1995) Intercellular communication and carcinogenesis. Mutat Res 333(1–2):181–188
18. Trosko JE, Ruch RJ (1998) Cell-cell communication in carcinogenesis. Front Biosci 3:d208–d236
19. Djalilian AR, McGaughey D, Patel S, Seo EY, Yang C, Cheng J, Tomic M, Sinha S, Ishida-Yamamoto A, Segre JA (2006) Connexin 26 regulates epidermal barrier and wound remodeling and promotes psoriasiform response. J Clin Invest 116(5):1243–1253
20. Langlois S, Maher AC, Manias JL, Shao Q, Kidder GM, Laird DW (2007) Connexin levels regulate keratinocyte differentiation in the epidermis. J Biol Chem 282(41):30171–30180
21. Maass K, Ghanem A, Kim JS, Saathoff M, Urschel S, Kirfel G, Grummer R, Kretz M, Lewalter T, Tiemann K, Winterhager E, Herzog V, Willecke K (2004) Defective epidermal barrier in neonatal mice lacking the c-terminal region of connexin43. Mol Biol Cell 15(10):4597–4608
22. Krutovskikh V, Mazzoleni G, Mironov N, Omori Y, Aguelon AM, Mesnil M, Berger F, Partensky C, Yamasaki H (1994) Altered homologous and heterologous gap-junctional intercellular communication in primary human liver tumors associated with aberrant protein localization but not gene mutation of connexin 32. Int J Cancer 56(1):87–94
23. Uchida Y, Matsuda K, Sasahara K, Kawabata H, Nishioka M (1995) Immunohistochemistry of gap junctions in normal and diseased gastric mucosa of humans. Gastroenterology 109(5):1492–1496
24. Tsai H, Werber J, Davia MO, Edelman M, Tanaka KE, Melman A, Christ GJ, Geliebter J (1996) Reduced connexin 43 expression in high grade, human prostatic adenocarcinoma cells. Biochem Biophys Res Commun 227(1):64–69
25. King TJ, Fukushima LH, Hieber AD, Shimabukuro KA, Sakr WA, Bertram JS (2000) Reduced levels of connexin43 in cervical dysplasia: inducible expression in a cervical carcinoma cell line decreases neoplastic potential with implications for tumor progression. Carcinogenesis 21(6):1097–1109
26. Loewenstein WR, Kanno Y (1966) Intercellular communication and the control of tissue growth: lack of communication between cancer cells. Nature 209(5029):1248–1249
27. Tada J, Hashimoto K (1997) Ultrastructural localization of gap junction protein connexin 43 in normal human skin, basal cell carcinoma, and squamous cell carcinoma. J Cutan Pathol 24(10):628–635
28. Temme A, Buchmann A, Gabriel HD, Nelles E, Schwarz M, Willecke K (1997) High incidence of spontaneous and chemically induced liver tumors in mice deficient for connexin32. Curr Biol 7(9):713–716
29. Avanzo JL, Mesnil M, Hernandez-Blazquez FJ, Mackowiak II, Mori CM, da Silva TC, Oloris SC, Garate AP, Massironi SM, Yamasaki H, Dagli ML (2004) Increased susceptibility to

urethane-induced lung tumors in mice with decreased expression of connexin43. Carcinogenesis 25(10):1973–1982

30. Saunders MM, Seraj MJ, Li Z, Zhou Z, Winter CR, Welch DR, Donahue HJ (2001) Breast cancer metastatic potential correlates with a breakdown in homospecific and heterospecific gap junctional intercellular communication. Cancer Res 61(5):1765–1767
31. Yang J, Liu B, Wang Q, Yuan D, Hong X, Yang Y, Tao L (2011) Connexin 32 and its derived homotypic gap junctional intercellular communication inhibit the migration and invasion of transfected hela cells via enhancement of intercellular adhesion. Mol Med Rep 4(5):971–979
32. McLachlan E, Shao Q, Laird DW (2007) Connexins and gap junctions in mammary gland development and breast cancer progression. J Membr Biol 218(1–3):107–121
33. Li Z, Zhou Z, Donahue HJ (2008) Alterations in cx43 and ob-cadherin affect breast cancer cell metastatic potential. Clin Exp Metastasis 25(3):265–272
34. Li Z, Zhou Z, Welch DR, Donahue HJ (2008) Expressing connexin 43 in breast cancer cells reduces their metastasis to lungs. Clin Exp Metastasis 25(8):893–901
35. Rhee DY, Zhao XQ, Francis RJ, Huang GY, Mably JD, Lo CW (2009) Connexin 43 regulates epicardial cell polarity and migration in coronary vascular development. Development 136(18):3185–3193
36. Mehta PP, Bertram JS, Loewenstein WR (1986) Growth inhibition of transformed cells correlates with their junctional communication with normal cells. Cell 44(1):187–196
37. Jara PI, Boric MP, Saez JC (1995) Leukocytes express connexin 43 after activation with lipopolysaccharide and appear to form gap junctions with endothelial cells after ischemia-reperfusion. Proc Natl Acad Sci U S A 92(15):7011–7015
38. El-Sabban ME, Pauli BU (1991) Cytoplasmic dye transfer between metastatic tumor cells and vascular endothelium. J Cell Biol 115(5):1375–1382
39. Pollmann MA, Shao Q, Laird DW, Sandig M (2005) Connexin 43 mediated gap junctional communication enhances breast tumor cell diapedesis in culture. Breast Cancer Res 7(4):R522–R534
40. El-Sabban ME, Pauli BU (1994) Adhesion-mediated gap junctional communication between lung-metastatic cancer cells and endothelium. Invasion Metastasis 14(1–6):164–176
41. Elzarrad MK, Haroon A, Willecke K, Dobrowolski R, Gillespie MN, Al-Mehdi AB (2008) Connexin-43 upregulation in micrometastases and tumor vasculature and its role in tumor cell attachment to pulmonary endothelium. BMC Med 6:20
42. Ito A, Katoh F, Kataoka TR, Okada M, Tsubota N, Asada H, Yoshikawa K, Maeda S, Kitamura Y, Yamasaki H, Nojima H (2000) A role for heterologous gap junctions between melanoma and endothelial cells in metastasis. J Clin Invest 105(9):1189–1197
43. Naoi Y, Miyoshi Y, Taguchi T, Kim SJ, Arai T, Maruyama N, Tamaki Y, Noguchi S (2008) Connexin26 expression is associated with aggressive phenotype in human papillary and follicular thyroid cancers. Cancer Lett 262(2):248–256
44. Ezumi K, Yamamoto H, Murata K, Higashiyama M, Damdinsuren B, Nakamura Y, Kyo N, Okami J, Ngan CY, Takemasa I, Ikeda M, Sekimoto M, Matsuura N, Nojima H, Monden M (2008) Aberrant expression of connexin 26 is associated with lung metastasis of colorectal cancer. Clin Cancer Res 14(3):677–684
45. Inose T, Kato H, Kimura H, Faried A, Tanaka N, Sakai M, Sano A, Sohda M, Nakajima M, Fukai Y, Miyazaki T, Masuda N, Fukuchi M, Kuwano H (2009) Correlation between connexin 26 expression and poor prognosis of esophageal squamous cell carcinoma. Ann Surg Oncol 16(6):1704–1710
46. Naoi Y, Miyoshi Y, Taguchi T, Kim SJ, Arai T, Tamaki Y, Noguchi S (2007) Connexin26 expression is associated with lymphatic vessel invasion and poor prognosis in human breast cancer. Breast Cancer Res Treat 106(1):11–17
47. Lim PK, Bliss SA, Patel SA, Taborga M, Dave MA, Gregory LA, Greco SJ, Bryan M, Patel PS, Rameshwar P (2011) Gap junction-mediated import of microrna from bone marrow stro-

mal cells can elicit cell cycle quiescence in breast cancer cells. Cancer Res 71(5):1550–1560

48. Ao M, Franco OE, Park D, Raman D, Williams K, Hayward SW (2007) Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. Cancer Res 67(9):4244–4253
49. Thery C, Ostrowski M, Segura E (2009) Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 9(8):581–593
50. Simons M, Raposo G (2009) Exosomes–vesicular carriers for intercellular communication. Curr Opin Cell Biol 21(4):575–581
51. Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH (2009) Exosomal microrna: a diagnostic marker for lung cancer. Clin Lung Cancer 10(1):42–46
52. Suetsugu A, Honma K, Saji S, Moriwaki H, Ochiya T, Hoffman RM (2013) Imaging exosome transfer from breast cancer cells to stroma at metastatic sites in orthotopic nude-mouse models. Adv Drug Deliv Rev 65(3):383–390
53. Balaj L, Lessard R, Dai L, Cho YJ, Pomeroy SL, Breakefield XO, Skog J (2011) Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nat Commun 2:180
54. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO (2007) Exosome-mediated transfer of mrnas and micrornas is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9(6):654–659
55. Xiao D, Ohlendorf J, Chen Y, Taylor DD, Rai SN, Waigel S, Zacharias W, Hao H, McMasters KM (2012) Identifying mrna, microrna and protein profiles of melanoma exosomes. PLoS One 7(10), e46874
56. Kogure T, Lin WL, Yan IK, Braconi C, Patel T (2011) Intercellular nanovesicle-mediated microrna transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology 54(4):1237–1248
57. Staubach S, Razawi H, Hanisch FG (2009) Proteomics of muc1-containing lipid rafts from plasma membranes and exosomes of human breast carcinoma cells mcf-7. Proteomics 9(10):2820–2835
58. Hood JL, Pan H, Lanza GM, Wickline SA, Consortium for Translational Research in Advanced I, Nanomedicine (2009) Paracrine induction of endothelium by tumor exosomes. Lab Invest 89(11):1317–1328
59. Tadokoro H, Umezu T, Ohyashiki K, Hirano T, Ohyashiki JH (2013) Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. J Biol Chem 288(48):34343–34351
60. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D (2012) Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through met. Nat Med 18(6):883–891
61. Gu J, Qian H, Shen L, Zhang X, Zhu W, Huang L, Yan Y, Mao F, Zhao C, Shi Y, Xu W (2012) Gastric cancer exosomes trigger differentiation of umbilical cord derived mesenchymal stem cells to carcinoma-associated fibroblasts through tgf-beta/smad pathway. PLoS One 7(12), e52465
62. Luga V, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, Buchanan M, Hosein AN, Basik M, Wrana JL (2012) Exosomes mediate stromal mobilization of autocrine wnt-pcp signaling in breast cancer cell migration. Cell 151(7):1542–1556
63. Abusamra AJ, Zhong Z, Zheng X, Li M, Ichim TE, Chin JL, Min WP (2005) Tumor exosomes expressing fas ligand mediate cd8+ t-cell apoptosis. Blood Cells Mol Dis 35(2):169–173

64. Chen WX, Liu XM, Lv MM, Chen L, Zhao JH, Zhong SL, Ji MH, Hu Q, Luo Z, Wu JZ, Tang JH (2014) Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of micrornas. PLoS One 9(4), e95240
65. Taylor DD, Gercel-Taylor C (2008) Microrna signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 110(1):13–21
66. Rosell R, Wei J, Taron M (2009) Circulating microrna signatures of tumor-derived exosomes for early diagnosis of non-small-cell lung cancer. Clin Lung Cancer 10(1):8–9
67. Silva J, Garcia V, Rodriguez M, Compte M, Cisneros E, Veguillas P, Garcia JM, Dominguez G, Campos-Martin Y, Cuevas J, Pena C, Herrera M, Diaz R, Mohammed N, Bonilla F (2012) Analysis of exosome release and its prognostic value in human colorectal cancer. Genes Chromosomes Cancer 51(4):409–418
68. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, Molinari A, Colone M, Tatti M, Sargiacomo M, Fais S (2009) Microenvironmental ph is a key factor for exosome traffic in tumor cells. J Biol Chem 284(49):34211–34222
69. Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, Zhang J, Weitz J, Chin L, Futreal A, Kalluri R (2014) Identification of double-stranded genomic DNA spanning all chromosomes with mutated kras and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem 289(7):3869–3875
70. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A, Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D (2014) Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 24:766–769. doi:10.1038/cr.2014.44
71. Bettermann K, Vucur M, Haybaeck J, Koppe C, Janssen J, Heymann F, Weber A, Weiskirchen R, Liedtke C, Gassler N, Muller M, de Vos R, Wolf MJ, Boege Y, Seleznik GM, Zeller N, Erny D, Fuchs T, Zoller S, Cairo S, Buendia MA, Prinz M, Akira S, Tacke F, Heikenwalder M, Trautwein C, Luedde T (2010) Tak1 suppresses a nemo-dependent but nf-kappab-independent pathway to liver cancer. Cancer Cell 17(5):481–496
72. Rana S, Malinowska K, Zoller M (2013) Exosomal tumor microrna modulates premetastatic organ cells. Neoplasia 15(3):281–295
73. Hood JL, San RS, Wickline SA (2011) Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res 71(11):3792–3801
74. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
75. Ciravolo V, Huber V, Ghedini GC, Venturelli E, Bianchi F, Campiglio M, Morelli D, Villa A, Della Mina P, Menard S, Filipazzi P, Rivoltini L, Tagliabue E, Pupa SM (2012) Potential role of her2-overexpressing exosomes in countering trastuzumab-based therapy. J Cell Physiol 227(2):658–667
76. Rasanen K, Vaheri A (2010) Activation of fibroblasts in cancer stroma. Exp Cell Res 316(17):2713–2722
77. Xu J, Lamouille S, Derynck R (2009) Tgf-beta-induced epithelial to mesenchymal transition. Cell Res 19(2):156–172
78. Lo HW, Hsu SC, Xia W, Cao X, Shih JY, Wei Y, Abbruzzese JL, Hortobagyi GN, Hung MC (2007) Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of twist gene expression. Cancer Res 67(19):9066–9076
79. Elliott BE, Hung WL, Boag AH, Tuck AB (2002) The role of hepatocyte growth factor (scatter factor) in epithelial-mesenchymal transition and breast cancer. Can J Physiol Pharmacol 80(2):91–102
80. Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, Oberyszyn TM, Hall BM (2009) Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. Oncogene 28(33):2940–2947

81. Fernando RI, Castillo MD, Litzinger M, Hamilton DH, Palena C (2011) Il-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells. Cancer Res 71(15):5296–5306
82. Nieto MA, Cano A (2012) The epithelial-mesenchymal transition under control: global programs to regulate epithelial plasticity. Semin Cancer Biol 22(5–6):361–368
83. Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139(5):871–890
84. Fuxe J, Vincent T, Garcia de Herreros A (2010) Transcriptional crosstalk between tgf-beta and stem cell pathways in tumor cell invasion: role of emt promoting smad complexes. Cell Cycle 9(12):2363–2374
85. Radisky DC (2005) Epithelial-mesenchymal transition. J Cell Sci 118(Pt 19):4325–4326
86. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119(6):1420–1428
87. De Craene B, Berx G (2013) Regulatory networks defining emt during cancer initiation and progression. Nat Rev Cancer 13(2):97–110
88. Akhurst RJ, Derynck R (2001) Tgf-beta signaling in cancer–a double-edged sword. Trends Cell Biol 11(11):S44–S51
89. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133(4):704–715
90. Paget S (1989) The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev 8(2):98–101
91. Balkwill F (2004) Cancer and the chemokine network. Nat Rev Cancer 4(7):540–550
92. Zlotnik A, Yoshie O (2012) The chemokine superfamily revisited. Immunity 36(5):705–716
93. Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, Lipp M (1999) Ccr7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99(1):23–33
94. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410(6824):50–56
95. Zlotnik A (2006) Involvement of chemokine receptors in organ-specific metastasis. Contrib Microbiol 13:191–199
96. Constantin G, Majeed M, Giagulli C, Piccio L, Kim JY, Butcher EC, Laudanna C (2000) Chemokines trigger immediate beta2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow. Immunity 13(6):759–769
97. Gupta GP, Massague J (2006) Cancer metastasis: building a framework. Cell 127(4):679–695
98. Fidler IJ (2003) The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 3(6):453–458
99. Witz IP (2008) The selectin-selectin ligand axis in tumor progression. Cancer Metastasis Rev 27(1):19–30
100. Laubli H, Spanaus KS, Borsig L (2009) Selectin-mediated activation of endothelial cells induces expression of ccl5 and promotes metastasis through recruitment of monocytes. Blood 114(20):4583–4591
101. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA (2007) Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449(7162):557–563
102. Velasco-Velazquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, Pestell RG (2012) Ccr5 antagonist blocks metastasis of basal breast cancer cells. Cancer Res 72(15):3839–3850
103. Keeley EC, Mehrad B, Strieter RM (2011) Chemokines as mediators of tumor angiogenesis and neovascularization. Exp Cell Res 317(5):685–690

104. Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, Buechi L, Walz A, Richmond A, Strieter RM (2000) The cxc chemokine receptor 2, cxcr2, is the putative receptor for elr + cxc chemokine-induced angiogenic activity. J Immunol 165(9):5269–5277
105. Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ (2000) Human endothelial cells express ccr2 and respond to mcp-1: direct role of mcp-1 in angiogenesis and tumor progression. Blood 96(1):34–40
106. Li X, Loberg R, Liao J, Ying C, Snyder LA, Pienta KJ, McCauley LK (2009) A destructive cascade mediated by ccl2 facilitates prostate cancer growth in bone. Cancer Res 69(4):1685–1692
107. Singh S, Varney M, Singh RK (2009) Host cxcr2-dependent regulation of melanoma growth, angiogenesis, and experimental lung metastasis. Cancer Res 69(2):411–415
108. Nathanson SD (2003) Insights into the mechanisms of lymph node metastasis. Cancer 98(2):413–423
109. Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo K, Claffey K, Detmar M (2001) Induction of tumor lymphangiogenesis by vegf-c promotes breast cancer metastasis. Nat Med 7(2):192–198
110. Oh SJ, Jeltsch MM, Birkenhager R, McCarthy JE, Weich HA, Christ B, Alitalo K, Wilting J (1997) Vegf and vegf-c: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane. Dev Biol 188(1):96–109
111. Saeki H, Moore AM, Brown MJ, Hwang ST (1999) Cutting edge: secondary lymphoid-tissue chemokine (slc) and cc chemokine receptor 7 (ccr7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 162(5):2472–2475
112. Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, Vestweber D, Corada M, Molendini C, Dejana E, McDonald DM (2007) Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 204(10):2349–2362
113. Hopken UE, Foss HD, Meyer D, Hinz M, Leder K, Stein H, Lipp M (2002) Up-regulation of the chemokine receptor ccr7 in classical but not in lymphocyte-predominant hodgkin disease correlates with distinct dissemination of neoplastic cells in lymphoid organs. Blood 99(4):1109–1116
114. Liu F, Lang R, Wei J, Fan Y, Cui L, Gu F, Guo X, Pringle GA, Zhang X, Fu L (2009) Increased expression of sdf-1/cxcr4 is associated with lymph node metastasis of invasive micropapillary carcinoma of the breast. Histopathology 54(6):741–750
115. Ding Y, Shimada Y, Maeda M, Kawabe A, Kaganoi J, Komoto I, Hashimoto Y, Miyake M, Hashida H, Imamura M (2003) Association of cc chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. Clin Cancer Res 9(9):3406–3412
116. Manzo A, Bugatti S, Caporali R, Prevo R, Jackson DG, Uguccioni M, Buckley CD, Montecucco C, Pitzalis C (2007) Ccl21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis. Am J Pathol 171(5):1549–1562
117. Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA (2007) Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine ccr7 signaling. Cancer Cell 11(6):526–538
118. Wiley HE, Gonzalez EB, Maki W, Wu MT, Hwang ST (2001) Expression of cc chemokine receptor-7 and regional lymph node metastasis of b16 murine melanoma. J Natl Cancer Inst 93(21):1638–1643
119. Uchida D, Onoue T, Begum NM, Kuribayashi N, Tomizuka Y, Tamatani T, Nagai H, Miyamoto Y (2009) Vesnarinone downregulates cxcr4 expression via upregulation of kruppel-like factor 2 in oral cancer cells. Mol Cancer 8:62
120. Mirisola V, Zuccarino A, Bachmeier BE, Sormani MP, Falter J, Nerlich A, Pfeffer U (2009) Cxcl12/sdf1 expression by breast cancers is an independent prognostic marker of disease-free and overall survival. Eur J Cancer 45(14):2579–2587

121. Salmaggi A, Maderna E, Calatozzolo C, Gaviani P, Canazza A, Milanesi I, Silvani A, DiMeco F, Carbone A, Pollo B (2009) Cxcl12, cxcr4 and cxcr7 expression in brain metastases. Cancer Biol Ther 8(17):1608–1614
122. Salogni L, Musso T, Bosisio D, Mirolo M, Jala VR, Haribabu B, Locati M, Sozzani S (2009) Activin a induces dendritic cell migration through the polarized release of cxc chemokine ligands 12 and 14. Blood 113(23):5848–5856
123. Sozzani S, Allavena P, D'Amico G, Luini W, Bianchi G, Kataura M, Imai T, Yoshie O, Bonecchi R, Mantovani A (1998) Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. J Immunol 161(3):1083–1086
124. Xu Q, Yuan X, Xu M, McLafferty F, Hu J, Lee BS, Liu G, Zeng Z, Black KL, Yu JS (2009) Chemokine cxc receptor 4--mediated glioma tumor tracking by bone marrow--derived neural progenitor/stem cells. Mol Cancer Ther 8(9):2746–2753
125. Sugiyama T, Kohara H, Noda M, Nagasawa T (2006) Maintenance of the hematopoietic stem cell pool by cxcl12-cxcr4 chemokine signaling in bone marrow stromal cell niches. Immunity 25(6):977–988
126. Cardones AR, Murakami T, Hwang ST (2003) Cxcr4 enhances adhesion of b16 tumor cells to endothelial cells in vitro and in vivo via beta (1) integrin. Cancer Res 63(20):6751–6757
127. Kim M, Koh YJ, Kim KE, Koh BI, Nam DH, Alitalo K, Kim I, Koh GY (2010) Cxcr4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. Cancer Res 70(24):10411–10421
128. Arigami T, Natsugoe S, Uenosono Y, Yanagita S, Arima H, Hirata M, Ishigami S, Aikou T (2009) Ccr7 and cxcr4 expression predicts lymph node status including micrometastasis in gastric cancer. Int J Oncol 35(1):19–24
129. Tanaka M, Grossman HB (2001) Connexin 26 gene therapy of human bladder cancer: induction of growth suppression, apoptosis, and synergy with cisplatin. Hum Gene Ther 12(18):2225–2236
130. Shishido SN, Nguyen TA (2012) Gap junction enhancer increases efficacy of cisplatin to attenuate mammary tumor growth. PLoS One 7(9), e44963
131. Li Z, Zhao R, Wu X, Sun Y, Yao M, Li J, Xu Y, Gu J (2005) Identification and characterization of a novel peptide ligand of epidermal growth factor receptor for targeted delivery of therapeutics. FASEB J 19(14):1978–1985
132. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, Gotoh N, Kuroda M (2013) Systemically injected exosomes targeted to egfr deliver antitumor microrna to breast cancer cells. Mol Ther 21(1):185–191
133. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G (2014) A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials 35(7):2383–2390
134. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K (2007) Molecular definition of breast tumor heterogeneity. Cancer Cell 11(3):259–273
135. Bhola NE, Balko JM, Dugger TC, Kuba MG, Sanchez V, Sanders M, Stanford J, Cook RS, Arteaga CL (2013) Tgf-beta inhibition enhances chemotherapy action against triple-negative breast cancer. J Clin Invest 123(3):1348–1358
136. Domanska UM, Timmer-Bosscha H, Nagengast WB, Oude Munnink TH, Kruizinga RC, Ananias HJ, Kliphuis NM, Huls G, De Vries EG, de Jong IJ, Walenkamp AM (2012) Cxcr4 inhibition with amd3100 sensitizes prostate cancer to docetaxel chemotherapy. Neoplasia 14(8):709–718