

Review Article

Roles for growth factors and mutations in metastatic dissemination

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Cancer is initiated largely by specific cohorts of genetic aberrations, which are generated by mutagens and often mimic active growth factor receptors, or downstream effectors. Once initiated cells outgrow and attract blood vessels, a multi-step process, called metastasis, disseminates cancer cells primarily through vascular routes. The major steps of the metastatic cascade comprise intravasation into blood vessels, circulation as single or collectives of cells, and eventual colonization of distant organs. Herein, we consider metastasis as a multi-step process that seized principles and molecular players employed by physiological processes, such as tissue regeneration and migration of neural crest progenitors. Our discussion contrasts the irreversible nature of mutagenesis, which establishes primary tumors, and the reversible epigenetic processes (e.g. epithelial–mesenchymal transition) underlying the establishment of micro-metastases and secondary tumors. Interestingly, analyses of sequencing data from untreated metastases inferred depletion of putative driver mutations among metastases, in line with the pivotal role played by growth factors and epigenetic processes in metastasis. Conceivably, driver mutations may not confer the same advantage in the microenvironment of the primary tumor and of the colonization site, hence phenotypic plasticity rather than rigid cellular states hardwired by mutations becomes advantageous during metastasis. We review the latest reported examples of growth factors harnessed by the metastatic cascade, with the goal of identifying opportunities for anti-metastasis interventions. In summary, because the overwhelming majority of cancer-associated deaths are caused by metastatic disease, understanding the complexity of metastasis, especially the roles played by growth factors, is vital for preventing, diagnosing and treating metastasis.

Introduction

The overwhelming majority of cancer-associated deaths (>90%) are caused by metastatic disease, rather than the respective primary tumors [1,2]. Hence, understanding the complexity of this process is vital for optimizing the treatment of patients with advanced malignancies. Already in 1889, Stephen Paget proposed that metastasis depends on cross-talk between cancer cells (the ‘seeds’) and specific organ microenvironments (the ‘soil’). However, despite intensive and prolonged research, the multi-step process of metastasis (often called the ‘invasion-metastasis cascade’ [3]) (Figure 1) remains poorly understood. This is in sharp contrast with the preceding steps of cancer progression, such as tumor initiation and primary tumor growth. Stated differently, while it is clear that somatically acquired ‘driver’ mutations, which affect oncogenes or tumor suppressor genes, propel the initial steps of malignancy, and their stepwise accumulation dictates the pace of tumor progression [4], the effects of genome aberration on metastasis appear less prominent. Instead, studies performed in the last two decades have highlighted control by epigenetic and other reversible processes, such as epithelial–mesenchymal transition (EMT), chemotaxis and a plethora of reciprocal (paracrine) cell-to-cell interactions regulated by soluble factors [5]. Herein, we consider the metastasis cascade as a multi-step process that seized the major principles and main molecular players employed by physiological rather

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than pathological processes. Such processes include reactions underlying tissue regeneration in wound healing and migration of neural crest (NC) progenitors in development. Our discussion is most relevant to the metastasis of carcinomas, which account for the majority of cancer mortality. We conclude by contrasting the irreversible nature of mutagenesis, which establishes primary tumors, and the phenotypically plastic and reversible processes underlying the establishment of micro-metastases and secondary tumors.

Principles borrowed from tissue regeneration

The major steps of the metastasis cascade comprise dissemination of cancer cells from the tumor of origin, subsequent intravasation into blood and lymph vessels, and eventual colonization of distant organs. Thus, the opening steps require breaching the basement membrane, which underlies large clusters of initiated cancer cells harboring pioneer mutations. Prior to this step, the primary tumor is considered a non-malignant lesion. For example, ductal carcinoma *in situ* (DCIS) is non-invasive breast cancer that encompasses a wide spectrum of diseases [6]. DCIS is characterized histologically by the rapid proliferation of initiated epithelial cells that are still bounded by the basement membrane of the breast ducts. Once epithelial integrity breaks and the underlying basement membrane is damaged, the surrounding tissue responds by activating a regenerative cascade analogous to the reaction of the skin to mechanical damage. A well-characterized marker of damaged epithelial progenitors is LICAM, which is required for regeneration of the epithelium and whose abundance in primary tumors is associated with poor disease outcome [7]. Natural wound healing proceeds through several phases involving an inflammatory response and associated cellular migration, proliferation, matrix deposition and tissue remodeling [8]. Activin and other members of the transforming growth factor beta (TGF- β) family play pivotal roles in both wound healing and cancer progression [9]. These processes are enabled, in part, by the ability of activin to control fibroblast proliferation and migration, as well as regulate the production of extracellular matrix (ECM) proteins and collagen cross-linking. In addition to fibroblasts and keratinocytes, macrophages, neutrophils and platelets are attracted to the wound and secrete EGF, VEGF, IL-1 and TGF- β , among other cytokines. In a similar way, the reactive stroma of invasive carcinomas releases various signals and displays markers of immune responses, as well as hypoxic responses [10]. As an outcome of hypoxia, blood vessels are attracted to invasive clusters of cells, such that angiogenesis is a major hallmark shared by both advanced tumors and deep wounds.

Subversion of progenitor migration during embryogenesis

Although hematogenous dissemination of cancer cells migrating within vascular channels is the best-studied route of metastatic dissemination, alternative pathways of distant colonization exist, but their research is hampered by technical issues. For example, cancer cells can migrate along nerves in a process called perineural invasion, which requires neoplastic invasion of nerves. The presence of cancer cells in the perineurium has been associated with poor prognosis, metastasis to lymph nodes and high recurrence of colorectal and other tumors [11,12]. Similarly, the abluminal surface of lymphatic and blood vessels (angiotropism) enables the spread of melanoma via extravascular migratory routes, while avoiding intravasation into vascular routes [13]. A recent report made use of an oncogenic BRAF mutation in mice with transgenic HGF overexpression and an oncogenic CDK4 germline mutation [14]. Interestingly, the authors reported that HGF-MET signaling and oncogenic BRAF can collaborate by enhancing angiotropic growth at the invasive front of primary tumors and in metastatic lesions of the lung. Notably, both perineural invasion and the extravascular migratory route avoid the shear force experienced by tumor cells, which is characteristic to hematogenous dissemination.

Unlike hematogenous dissemination, extravascular migration of embryonic progenitors widely occurs in morphogenesis. Perhaps the best understood and the one most relevant to metastasis is the migration of NC cells in the developing embryo [15]. The NC is a highly migratory embryonic cell population that develops into numerous cell lineages, including melanocytes, smooth muscles, neurons and the craniofacial mesenchyme. Importantly, NC cells migrate along well-defined routes in the developing embryo and display an enormous ability to invade tissues and organs. After the NC forms along the border of the neural plate, NC cells undergo EMT, whereby polarized epithelial cells lose adhesion and adopt mesenchymal morphologies in preparation for migration [16]. Many of the processes underlying the formation and migration of NC cells can also play critical roles in cancer progression [17]. For example, both NC cells and circulating tumor cells (CTCs) form loosely associated collectives and use both guidance cues and the local ECM to collectively migrate. In breast cancer, collective invasion represents the predominant invasion mode and associates with distant metastasis [18]. The use of mouse models with tagged mammary tumors provided important insights: although rare in the

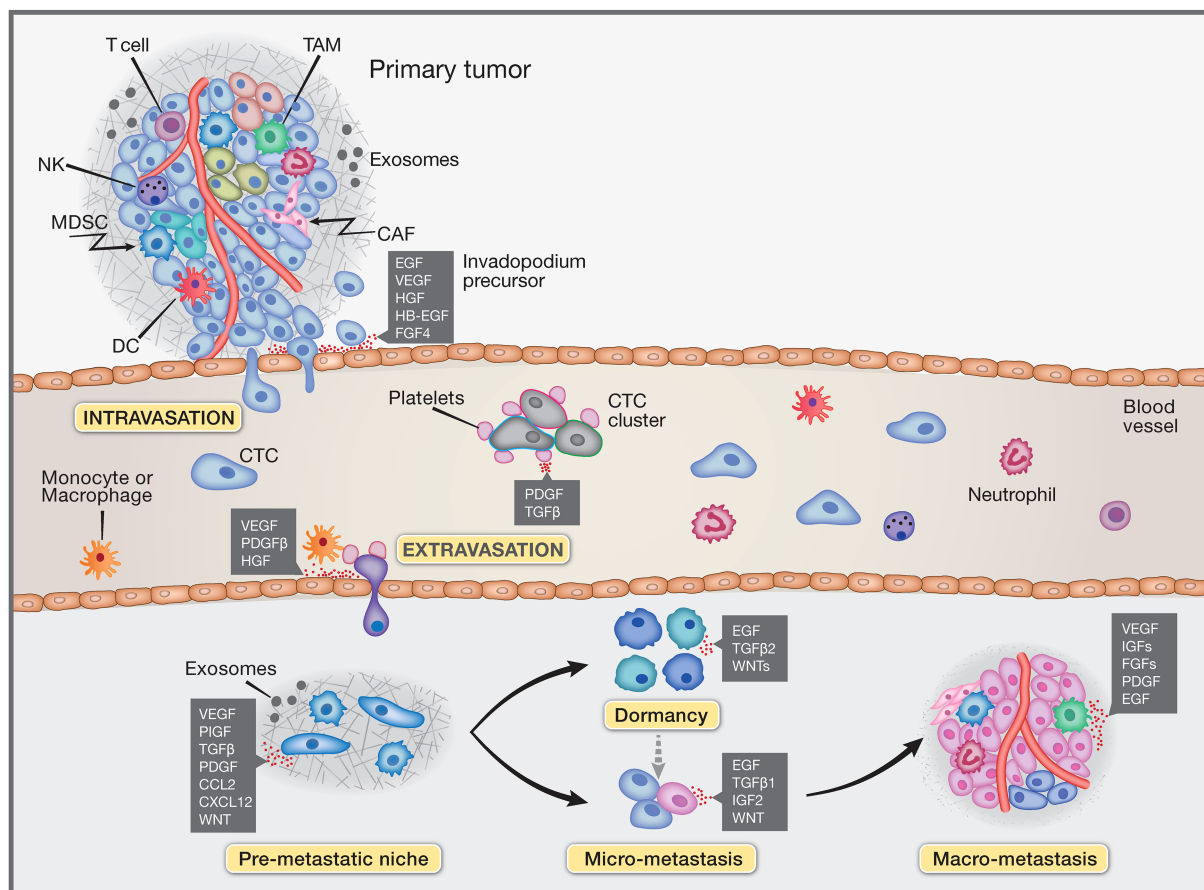


Figure 1. Schematic model of the metastatic spread of cancer cells.

Primary tumor cells are embedded in a microenvironment comprising stromal cells, such as cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSC), dendritic cells (DCs), natural killer (NK) cells, T cells, tumor-associated macrophages (TAMs), neutrophils, along with blood vessels. Shedding of tumor cells and their invasion across tissue barriers permit establishment of circulating tumor cells (CTCs) and complete the process called intravasation. While in blood vessels, CTC clusters and solitary cells are protected by platelets and neutrophils, which facilitate extravasation. Tumor-secreted soluble factors, tumor-shed extracellular vesicles (exosomes) and bone marrow-derived cells (BMDCs) co-operate to form the pre-metastatic niche. Micro-metastases often experience a variable length dormancy phase, which might lead to cancer cell death. An angiogenic switch can initiate outgrowth and formation of secondary tumors. Growth factors (shown as red dots) and cytokines involved in each step of the cascade are indicated in the corresponding boxes. The abbreviations used are: CCL2, C-C motif chemokine ligand 2; CXCL12, C-X-C motif chemokine ligand 12; EGF, epidermal growth factor; FGF, fibroblast growth factor; HB-EGF, heparin-binding EGF-like growth factor; HGF, hepatocyte growth factor; IGF, insulin like growth factor; PDGF, platelet-derived growth factor; PIGF, placental growth factor; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor; WNTs, Wnt family members.

circulation compared with single CTCs, CTC clusters have up to 50-fold increased metastatic potential [19]. These studies also identified the cell junction component plakoglobin as highly differentially expressed, such that knockdown of plakoglobin abrogated CTC cluster formation and suppressed lung metastases.

Cell-to-cell interactions while CTCs are traveling in the circulation

While in transit, CTCs are vulnerable to immune attacks, hydrodynamic flow and shear stress, but interactions with specific cells, especially platelets, neutrophils, monocytes and endothelial cells, might support their survival and facilitate eventual extravasation [1]. First noted by the French physician Armand Trousseau, increased incidence of venous thrombosis associated with certain cancers (Trousseau's syndrome) [20]. The molecular basis

of Trousseau's syndrome has been attributed to the ability of platelets to protect tumor cells in circulation from the normal immune response or natural killer cells [21]. In addition, platelet-derived lysophosphatidic acid (LPA) can support and activate metastatic breast cancer cells, as well as stimulate the release from cancer cells of interleukin-6 and interleukin-8, leading to bone destruction and further supporting metastatic growth [22]. Of note, LPA is a natural bioactive lipid with growth factor-like functions due to activation of a series of six G protein-coupled receptors (LPA₁₋₆). LPA receptor type 1 (LPA₁) signaling has been associated with the up-regulation of heparin-binding EGF-like growth factor (HB-EGF) [23]. Accordingly, analysis of primary tumors of a large cohort of breast cancer patients found a significantly higher expression of HB-EGF in breast tumors expressing high levels of LPA₁. These results raise the possibility of using anti-HB-EGF antibodies or LPA₁ antagonists to inhibit metastasis.

Although neutrophils — the most abundant leukocytes in human blood — can kill disseminated cancer cells under certain conditions, CTCs can recruit neutrophils to promote metastasis. For instance, neutrophils can enhance the extravasation of tumor cells, mainly through the secretion of matrix metalloproteinases [24]. One way enabling neutrophils to kill harmful microorganisms entails the formation of neutrophil extracellular traps (NETs), which are DNA meshes released into the extracellular space, where they trap microorganisms. NETs form in human pancreatic, liver and gastric cancer, as well as within the vasculature. In animal models, NET-like structures form around metastatic cells and stimulate the invasion and migration of breast cancer cells, implying that induction of NETs by cancer cells promotes metastasis [25]. Importantly, G-CSF, a key regulator of neutrophil production and a potent mobilizer of haemopoietic stem cells, emerged as a critical factor in the induction of NETs by cancer cells.

Cancer cell extravasation

Cancer cell extravasation usually occurs in small capillaries, where CTCs are physically trapped by size restriction. Once pressed into small capillaries, CTCs are deformed and undergo mechanical stress that is responsible for the loss of up to 90% of cancer cells entering small vessels [26]. Nevertheless, subsets of cancer cells eventually infiltrate the parenchyma of organs to seed metastatic colonies. The underlying process, termed transendothelial migration (TEM), depends on disruptors of vascular integrity, such as VEGF, metalloproteinases and many pairs of ligand–receptor molecules, such as selectins, integrins, cadherins and CD44. In addition, a distinct population of host macrophages is recruited to metastatic cells and enables efficient tumor cell extravasation [27]. Several studies revealed that local death of endothelial cells can enhance extravasation, for example by means of ATP released by dying cells. It has been shown that the release of ATP can enhance the survival of tumor cells in the face of mechanical stress [28]. Interestingly, human and murine tumor cells induce programmed necrosis (necroptosis) of endothelial cells, which promotes tumor cell extravasation and metastasis [29]. Unexpectedly, tumor-cell-induced endothelial necroptosis requires amyloid precursor protein expressed by tumor cells and its receptor, death receptor 6 (DR6), on endothelial cells, as the primary mediators of necroptotic signaling.

Organ-specific metastatic potential and the pre-metastatic niche

It is widely accepted that metastasis is an inefficient process whereby the vast majority of CTCs are not able to survive and proliferate at distant sites. In addition, metastatic cells often display tissue and organ tropism, in a way that cannot be explained by circulatory patterns. Whether or not organ-specific metastasis potential is driven by the accumulation of genetic mutations or by epigenetic events, such as up-regulation of adhesion molecules or other factors, remains unclear [3]. For example, breast cancer metastases frequently colonize the bone and the lung, and less frequently the liver and brain. By means of sequential isolation of isogenic mammary tumor cells that preferentially infiltrate the brain, it became clear that cell-surface glycosylation dictates organ-specific metastatic interactions [30]. Along with other genes, *HB-EGF* emerged from this study as an enhancer of brain metastasis, as opposed to metastasis to liver or lymph nodes. Interestingly, another EGFR ligand, epiregulin, was found to be included in an 18-gene lung metastasis signature [31]. According to an alternative view, a favorable microenvironment, called the pre-metastatic niche (PMN), located in an organ distant from a primary tumor, is critical for tumor metastasis and target tissue selection. PMN establishment requires both extracellular vesicles (exosomes) [32] and bone marrow-derived haematopoietic progenitor cells. The latter cells express vascular endothelial growth factor receptor 1 (VEGFR1/Flt1) and form cellular clusters

before the arrival of tumor cells [33]. In addition to bone marrow-derived cells, cancer-derived factors, and ECM, a host of growth factors and pro-inflammatory cytokines are involved [34]. The list includes VEGF, WNT ligands, interleukin (IL)-6, IL-1 β , CC-chemokine ligand 2 (CCL2), granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), stromal cell-derived factor (SDF)-1, macrophage migration inhibitory factor (MIF) and Chemokine (C-X-C motif) ligand 1 (CXCL1; see boxes in Figure 1).

Metastasis-driving genetic aberrations versus phenotypic plasticity

In accord with the large contribution of metastasis to the incidence of oncology-related deaths, it has been reported that predictors of cellular migration, rather than proliferation of breast cancer cells, are strongly associated with patient survival [35]. Surprisingly, however, only very few driver mutations have been directly linked to the metastatic cascade. For example, a recent screen aimed at putative driver mutations escalating the risk of metastasis identified three mutations in nucleoporin 93 (NUP93), a component of nuclear core complexes [36], which is involved in 3D migration and reorganization of the actin cytoskeleton [37]. Guided by the principle that cancer, including metastasis, involves the evolution of malignant cells attempting to survive under the stresses of tumor progression [38], larger-scale surveys of cancer genomics have tried identifying pro-metastatic mutations [2]. Apparently, there are two general scenarios of metastatic dissemination, and both have been supported by genome-wide analyses: In the linear progression model, the metastasis-competent clone arises late in tumorigenesis and disseminates just before clinical detection of the primary lesion. Hence, the expected genetic divergence between the primary tumor and its metastasis (termed P-M genetic divergence) might be rather limited [38]. In the parallel progression model, the metastatic clone disseminates from the primary tumor early on, and both the primary tumor and the metastases continue to evolve in parallel. As a result, the P-M genetic divergence might be substantial. Sampling and exposure to systemic therapy might confound the inference of the exact mode of metastatic spread, especially because therapy frequently associates with adaptive mutagenesis [39]. To circumvent this, Reiter and colleagues analyzed sequencing data from untreated metastases and inferred cancer phylogenies [40]. Their analyses unveiled depletion of putative driver mutations among metastases, and the majority of those that were observed had only weak or no predicted functional effects. These observations are consistent with the ‘Big Bang’ model proposed by Christina Curtis and colleagues [41]. Accordingly, tumors grow predominantly as a single expansion producing many subclones that are not subject to stringent selection, and where both clonal and subclonal alterations arise early during growth.

Roles for EMT, MET and other epigenetic switches in metastasis

Several reasons might explain why inter-metastatic heterogeneity is relatively low, while paracrine loops involving growth factors are widespread. For one, under certain conditions, disseminated cancer cells enter proliferative quiescence, such that the tumor becomes dormant (e.g. due to failure to activate angiogenesis). In addition, driver gene mutations may not confer the same advantage in the microenvironment of the primary tumor and of a distant site. Stated differently, phenotypic plasticity and epigenetic events, rather than rigid cellular states hardwired by mutations, might become advantageous at the late stages of tumor progression. The metastasis cascade provides several examples of reversible changes. While the invasion and dissemination steps during carcinoma progression have been associated with EMT, the reverse process — mesenchymal-epithelial transition (MET) — likely propels the outgrowth of cancer cells once they settled at distant sites. For example, repression of the EMT inducer *Prrx1* is essential for metastatic colonization [42]. In similarity, activation of another EMT-inducing transcription factor, *Twist1*, is sufficient for stimulating carcinoma cells to undergo EMT. However, reversion of this process is essential for the proliferation of disseminating tumor cells at distant organs [43]. Notably, whether or not EMT is obligatory for metastasis remains a longstanding source of debate. For instance, inhibiting EMT by overexpressing the microRNA 200 did not affect spontaneous breast-to-lung metastasis [44], and mouse models of pancreatic ductal adenocarcinoma (PDAC) with deletion of *Snail* or *Twist* did not alter systemic dissemination or metastasis [45]. This might be explained by the transient nature of EMT or by the existence of a novel metastasis program, a possibility raised by experiments that made use of mesenchymal cell reporter mice and a PDAC model [46].

Viewed from a slightly different angle, emerging data show that EMT comprises a spectrum of intermediate states, coined as hybrid or partial EMT. Hybrid EMT cells exhibit high tumorigenic properties, leading to stemness and therapy resistance [47]. Therefore, it has been argued that these cells are more prone to colonize distant organs. Using a lineage-labeled mouse model of PDAC, Aiello et al. [48] studied EMT *in vivo* and reported that most tumors lose their epithelial phenotype to acquire the ‘partial EMT’ phenotype. Importantly, carcinoma cells utilizing this program migrate as clusters, which contrast with the single-cell migration pattern associated with EMT mechanisms. According to another report, adherens junctions and the EMT–MET interconversion play roles in metastatic organotropism of pancreatic cancer [49]. Furthermore, it has been observed that loss of FAT1, which encodes a protocadherin, increased tumor stemness and spontaneous metastasis, through the induction of a hybrid EMT state [50].

In conclusion, reversible EMT appears essential for tumor metastasis. Cellular plasticity extends to tumor-initiating stem cells and the ability to form metastases. For instance, hyperactivation of the WNT signaling pathway turns intestinal LGR5⁺ cells into tumor-initiating cancer stem cells (CSCs), but the cells that disseminate from the primary tumor and seed liver metastasis in mouse models are predominantly LGR5⁻ [51]. Still, once established in the liver, some proliferating metastatic cells re-acquire LGR5 expression. Apart from WNT, this pathway might be activated by EGF family growth factors [52] and other growth factors capable of stimulating SMAD3 [53], which demonstrates the versatility and plasticity of LGR5 induction and the metastatic cascade. To exemplify the scope and variety of the contribution of epigenetic switches and soluble factors to metastasis, below we describe several recent examples and provide a longer list of growth factors in Table 1.

Diverse mechanisms enable regulation of metastasis by growth factors

Growth factors regulate the biochemistry and mechanics of metastasis

Several actin-filled protrusions facilitate migration and invasion of cancer cells. They include lamellipodia and filopodia at the leading edge, and invadopodia facilitating invasion through the epithelium and basal membrane [54]. Invasion across tissue barriers requires cell softening, which is, surprisingly, preceded by transient accumulation of actin stress fibers and cell stiffening [55]. Invadopodia formation is regulated by growth factors and signals from the ECM [56,57]. Growth factor- and oncogene-activated cells are characterized by active PI3K (phosphoinositol 3-kinase) and elevated levels of the phosphoinositol lipid PI(3,4,5)P₃. Dephosphorylation of this lipid by 5'-phosphatases, such as synaptojanin 2 (SYNJ2), generates PI(3,4)P₂, which recruits to the plasma membrane an adaptor, TKS5, necessary for the nucleation of invadopodia. SYNJ2 is transcriptionally up-regulated on the treatment of mammary cells with EGF. In addition, SYNJ2 is encoded at 6q25, a chromosomal locus amplified in aggressive forms of breast cancer [58]. In line with this, several reports linked SYNJ2 to invasiveness of glioma and other cancer cells. Normally, two tumor suppressor phosphatases, PTEN and INPP4B, deplete PI(3,4)P₂ [59] and balance the oncogenic alliance formed by PI3K and SYNJ2. In summary, by regulating inositol lipids and the actin cytoskeleton, growth factors can enhance invasiveness during both intravasation and extravasation.

Clustering of CTCs is enhanced by growth factors

Clustering of CTCs confers an advantage in terms of successful colonization of distant organs, but the underlying mechanisms are incompletely understood. CTC aggregation depends on cell-to-cell adhesion molecules, such as plakoglobin, CD44 and E-cadherin [19]. According to a recent report, CTCs switch between proliferative and migratory states based on the concentration of a growth factor, epigen, found in nanoscale intercellular compartments present within the clusters [60]. Initially, when applying RNA-seq on tumor cells of different aggregation conditions, the authors found that epigen (along with amphiregulin) was the most induced gene upon tumor cell clustering. Furthermore, depletion of epigen using RNA interference indicated that this growth factor is not required for cell clustering but it might support metastatic outgrowth. Further analyses showed that epigen, a low-affinity ligand of EGFR, was highly concentrated in intercellular sealed cavities. Interestingly, similar principles might permit another family of growth factors to control morphogenesis during embryonic development: Durdu et al. [61] provided evidence for a model in which diffusible signals in the form of fibroblast growth factors (FGFs) are controlled by trapping FGF molecules within small, closed extracellular spaces (termed microlumina), from which they have access to only a discrete collection of cells. Future studies might

Table 1. Roles for the major growth factors in metastasis

Part 1 of 3

GF family	Receptors	Roles in metastasis
EGF and NRG	EGFR, HER2, HER3, HER4	Constitutive activation and overexpression of ERBB/HER receptors are correlated with poor prognosis, drug resistance, cancer metastasis, and shorter patient survival rates in several types of cancer [69,70].
EGF		<ul style="list-style-type: none"> EGF secreted by M2-like TAMs suppresses lncRNA LIMT expression via activating EGFR-ERK signaling pathway to promote ovarian cancer metastasis [71]. HER2 and EGFR promote prostate cancer metastasis to bone [72].
TGF α		<ul style="list-style-type: none"> TFCP2 induces TNBC progression via a positive feedback loop comprising EGF/TGFα and the AKT signaling axis [73]. A TNFα-TGFα-EGFR interacting loop between tumor and stromal cells promotes peritoneal metastasis of ovarian cancer [74].
NRG 1-4		<ul style="list-style-type: none"> Cross-talk between the Hippo–YAP pathway and the heterodimer kinase complex formed by ROR1 with HER3 promotes breast cancer bone metastasis in a lncRNA-dependent manner [75]. NRG1-HER4-YAP signaling contributes to migration of breast cancer cells [76]. NRG1 in luminal breast cancer defines pro-fibrotic and migratory CAFs [77]. Tumor microenvironment-derived NRG1 promotes antiandrogen resistance in prostate cancer [78]. AREG/EGFR signaling enhances the suppressive function of Treg cells and promotes tumor metastasis [79].
AREG		<ul style="list-style-type: none"> AREG overexpression induces a phenotypic switch from malignant ascites to solid metastatic phenotype in a cell clone obtained from a patient derived-ovarian cancer model [80].
EREG		<ul style="list-style-type: none"> Autocrine epiregulin contributes to lung metastasis via EMT in salivary adenoid cystic carcinoma through activation of EGFR [81]. EREG is essential for transformation of fibroblasts to CAFs, which is required to induce EMT and invasion via the JAK2-STAT3 pathway in OSCC [82].
HB-EGF		<ul style="list-style-type: none"> Inhibition of the HB-EGF/EGFR pathway using peptides inhibited HB-EGF-induced ovarian cancer cell migration and invasion [83].
EPIGEN		<ul style="list-style-type: none"> Tumor cell clusters produce epigen, which controls switching between collective migratory and proliferative modes. Epigen knockdown strongly reduces metastatic outgrowth [60].
TGF-β	TGF-βR1,2	TGF-β plays a dual role during tumorigenesis; in early stages of carcinogenesis, it induces growth inhibition, but in the late stages it promotes cancer progression and metastasis, due to its ability to dedifferentiate many cell types, suppress immune cell development and indirectly allow vascular growth [84,85].
TGF- β 1-3		<ul style="list-style-type: none"> TGF-β and MMPs produced by myeloid cells in advanced-stage cancer inhibit antitumor immune reactions and promote metastasis [86]. By repressing the expression of miR-211, adipocytes sensitize melanoma cells to TGF-β signaling, leading to a switch from a proliferative to an invasive phenotype [87]. EGFR, AP-1, p63, and TGF-β co-operate to promote invasiveness of breast cancers [88]. TGF-β-induced DACT1 biomolecular condensates repress WNT signaling to promote bone metastasis [89]. Activation of the canonical BMP4-SMAD7 signaling pathway blocks breast cancer metastasis [90].
BMPs		
VEGF	VEGFR1-3	VEGFs plays a role in tumor-associated angiogenesis, tissue infiltration, and metastasis formation [91,92].
VEGF-A		<ul style="list-style-type: none"> VEGF-A secreted by primary tumors causes vascular hyperpermeability in pre-metastatic lung via the occludin phosphorylation/ubiquitination pathway [93]. Suspending anti-VEGF therapy induces metastasis through a liver revascularization mechanism [94]. VEGF-A expression promotes fibrosarcoma metastasis [95].
VEGF-B		<ul style="list-style-type: none"> VEGF-B promotes cancer metastasis through the remodeling of tumor microvasculature [96].
VEGF-C		<ul style="list-style-type: none"> VEGF-C and FGF-2, collaboratively promote angiogenesis, lymphangiogenesis and lymph-node metastases [97]. By inducing EMT, epithelial breast cancer cell cross-talk, VEGF-C promotes tumor growth and metastasis [98]. VEGF-C up-regulation induced by c-MYC promotes lymphatic metastasis of pancreatic neuroendocrine tumors [99].
VEGF-D		<ul style="list-style-type: none"> Breast cancer cell subclones expressing IL-11 and FIGF (VEGF-D) promote formation of polyclonal metastases composed of driver and neutral subclones [100].

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Table 1. Roles for the major growth factors in metastasis

Part 2 of 3

GF family	Receptors	Roles in metastasis
IGF IGF1 IGF2	IGF1R, IGF2R	IGF signaling promotes cancer progression by affecting tumorigenesis, metastasis and resistance to cancer therapies [101,102]. <ul style="list-style-type: none"> • CAF-derived IGF-1 primes breast cancer cells for bone metastasis [103]. • CAFs induce invasion of PDAC cells through paracrine IGF1/IGF1R signaling [104]. • NDRG1 inhibits PSC-CM-induced migration of PaCa via inhibition of HGF/c-MET, IGF-1/IGF-1R signaling [105]. • IGF2 secreted by pericyte promotes formation of breast cancer brain metastasis [65].
HGF HGF	MET, RON	HGF/MET signaling promotes EMT and cancer invasiveness [106]. <ul style="list-style-type: none"> • Inhibition of HGF and MET together with chemotherapy prevented metastasis in a model of PaCa [107]. • HGF/MET controls EMT and metastasis via FOSL2 in NSCLC [108]. • HGF-mediated cross-talk between CAFs and gastric cancer cells activates metastasis [109]. • Breast cancer cells with high metastatic potential are hypersensitive to HGF secreted by macrophages [110]. • MET signaling activates an inflammatory microenvironment in the brain and facilitates breast cancer metastasis to the brain [111].
FGF FGF 1-23	FGFR1-4	FGFs are considered cancer drivers for their ability to regulate angiogenesis, cell proliferation and metastasis [112,113]. <ul style="list-style-type: none"> • Loss of TGF-β signaling in osteoblasts increases basic FGF to promote bone metastases in an animal prostate cancer model [114]. • Activation of FGF2/FGFR1 promotes cell proliferation, EMT and metastasis in FGFR1-amplified lung cancer via the FGFR1-ERK1/2-SOX2 axis [115]. • Collective migration of paraneoplastic cells is mediated by FGF signaling [116].
PDGF PDGF-AA PDGF-BB PDGF-CC PDGF-DD PDGF-A/B	PDGFRα,β	By regulating mesenchymal cells such as fibroblasts, pericytes and smooth muscle cells, PDGFs and their receptors can promote tumor metastasis [117,118]. <ul style="list-style-type: none"> • Loss of miRNA let-7d and gain of HIF1 activity promote breast cancer brain metastasis via PDGF; inhibition of PDGFR suppresses brain metastasis [119]. • In pericytes and stromal fibroblasts, the PDGF-BB-PDGFRβ-IL-33-ST2 axis recruits TAMs and induces metastasis [120]. • PDGF-C secreted by CAFs promotes GIST growth and metastasis [121]. • PDGF-BB induces pericyte-fibroblast transition (PFT), contributing to tumor invasion and metastasis [122].
CSF1 (M-CSF)	CSF1R	CSF1/CSF1R signaling indirectly promotes tumor progression by regulating the functions of macrophages, or directly, by promoting release of inflammatory mediators from tumor cells [123]. <ul style="list-style-type: none"> • Co-overexpression of TWIST1 and CSF1 promotes OSCC invasiveness [124]. • The Oct4/CSF1 axis promotes M2 macrophage polarization, leading to lung cancer growth and metastasis [125]. • miR-149 acts as a metastasis-suppressing microRNA in breast cancer cells by reducing recruitment and M2-polarization of macrophages induced by CSF1 [126].
WNT	FZDs Co-receptors: LRP 5/6 Ryk ROR 1/2	Both canonical and non-canonical WNT pathways in the tumor microenvironment contribute to EMT, metastasis and cancer stem cell maintenance in lung, colorectal and breast cancers [127]. <ul style="list-style-type: none"> • The WNT pathway acts as a driver of bone metastatic invasion of prostate cancer [128]. • The canonical WNT/β-catenin/Slug pathway contributes to cancer cell invasion and lymph-node metastasis of HNSCC [129]. • Autocrine WNT7b plays a role in CRC metastasis by promoting EMT through the WNT/β-catenin signaling pathway [130].
CXCL12	CXCR4	The CXCL12/CXCR4 axis regulates metastasis via different mechanisms (e.g. EMT and up-regulation of metalloproteinases) in several cancers, including pancreatic, colon and melanoma [131,132]. <ul style="list-style-type: none"> • The CXCR4-LASP1 axis enhances the stability of nuclear Snail1, to promote invasion of TNBC cells [133]. • CXCL12/CXCR4 promotes invasion of ovarian cancer cells by means of suppressing ARHGAP10 expression [134]. • CXCR4 signaling promotes the interaction between tumor cells and neutrophils, to regulate onset of metastasis [135].

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Table 1. Roles for the major growth factors in metastasis

Part 3 of 3

GF family	Receptors	Roles in metastasis
NGF	TrkA P75NTR	<ul style="list-style-type: none">• Inhibition of DPP-4 facilitates breast cancer metastasis by activating the CXCL12/CXCR4/mTOR axis and promoting EMT [136].• Cross-talk between fibroblasts derived CXCL12 and endothelial cells promotes tumor cell intravasation, leading to metastasis [137].• PDGFRα induces SCC metastasis to the lungs through induction and secretion of SDF-1 (CXCL12), with consequent activation of CXCL12/CXCR4 signaling [138].• NGF involved in the mediation of brain metastases and enhanced survival [139].
		<ul style="list-style-type: none">• The HDGF-ALCAM axis promotes metastasis of Ewing sarcoma by means of regulating GTPases [140].
HDGF		

Listed are the major classes of growth factors engaged in metastasis, their high-affinity receptors (primarily receptors harboring tyrosine kinase functions), and recently reported functions. The abbreviations used are listed below: CAFs, cancer-associated fibroblasts; EMT, epithelial–mesenchymal transition; GIST, gastrointestinal stromal tumors; HDGF, hepatoma-derived growth factor; HNSCC, head and neck squamous cell carcinoma; NGF, nerve growth factor; NSCLC, non-small cell lung cancer; PaCa, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; PSC-CM, pancreatic stellate cell-conditioned media; ROR1, receptor tyrosine kinase-like orphan receptor-1; SCC, squamous cell carcinoma; TAMs, tumor-associated macrophages; TNBC, triple-negative breast cancer.

resolve the question whether or not collectives of CTCs harness a developmental principle to gain improved colonization.

Loss of imprinting underlays driver roles of IGF2 in ileal neuroendocrine tumors

A recent, small intestine cancer study, provides an example of epigenetic regulation of metastasis (i.e. gene silencing). Ileal neuroendocrine tumors (I-NETs), which affect the distal portion of the small intestine, usually progress slowly, but they have often metastasized to the liver by the time the patient presents [62]. Due to robust and extensive metastasis, some patients with advanced I-NET undergo liver transplantation. The genetic driver of this disease has long remained a mystery. The overall frequency of mutations in I-NETs is rather low and I-NETs have no known mutations in oncogenes or tumor suppressor genes. Although no animal models of I-NETs have been available, the transgenic RT2 system [63], originally developed as a model of insulinomas, can model different NET subtypes if the genetic background is changed. It has recently been shown that RT2 mice can develop I-NETs on a specific genetic background. Analysis of this first I-NET animal model allowed identification of the insulin-like growth factor 2 (IGF2) as the first I-NET driver gene [64]. Elevated serum IGF2 is associated with an increased risk of developing various cancers, including colorectal. IGF2, as well as the receptor, IGF2R, is one of a relatively small number of imprinted genes in mammals. Thus, due to maternal imprinting, IGF2 is typically expressed from the paternal allele only. I-NETs generated by transgenic RT2 mice depended upon genetic background and displayed loss of imprinting (LOI) [64]. As would be expected, LOI correlated with increased *IGF2* transcription in the tumors of 57% of patients with I-NET. In summary, *IGF2* appears to be the first genetic driver of the highly metastatic but mutation-poor I-NET tumors. Importantly, LOI is expected to only double IGF2 concentrations, but this might be sufficient for tumorigenesis, which underscores the importance of growth factor homeostasis. Notably, the involvement of secreted IGF2 in metastasis has recently been reported by a study that analyzed brain pericytes producing IGF2 and promoting metastasis of breast cancer to brain [65].

Roles for TGF-beta in the establishment of the pre-metastatic niche

The pleiotropic actions of TGF- β as a mediator of immune homeostasis and tolerance, inducer of EMT and ECM, as well as a promoter of tumor immune evasion, complicate interpretation of the influence of the TGF- β pathway on cancer progression [66]. While in early stages this pathway has tumor suppressor functions, such as cell-cycle arrest and apoptosis, in late stages TGF- β can promote tumorigenesis, including metastasis. One exemplification of the late-stage roles and the ability of TGF- β to enhance metastasis has been linked to the PMN [67]. As aforementioned, PMN formation is a stepwise process resulting from the combined systemic effects of tumor-secreted factors and exosomes. For example, according to a recent report, a tumor-exosome

mediated pathway promotes liver metastasis of PDAC cells in a mechanism involving TGF- β and fibronectin [68]. The authors pre-treated naive mice with exosomes derived from normal pancreatic cells, or from PDAC cells, and later injected the PDAC cells into the portal vein of the mice to generate liver metastases. Mice pre-treated with PDAC-derived exosomes exhibited significantly more metastatic lesions in the liver, indicating that these exosomes can condition the liver towards a microenvironment able to support the growth of PDAC cells. Additional experiments revealed that uptake of PDAC-derived exosomes by Kupffer cells enhanced secretion of TGF- β and production of fibronectin by hepatic stellate cells. As a result, the newly formed fibrotic microenvironment enhanced the recruitment of bone marrow-derived macrophages. These findings suggest that tumor-derived exosomes prime the liver for metastasis.

Perspective

- Because the overwhelming majority of cancer-associated deaths are caused by metastatic disease, understanding the complexity of this process is vital to optimizing the treatment of patients with advanced malignancies.
- Metastasis is driven by a combination of genetic drivers (mutations) and a large variety of epigenetic processes conferring a diverse collection of phenotype alterations, which offer ample opportunities for pharmacological interventions.
- Precise understanding of the metastatic process and the multiple roles played by growth factors and signaling pathways will enable effective ways to prevent, diagnose and treat metastasis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

N.B.N., I.M. and Y.Y. collected data from scientific publications, designed the manuscript and wrote the text.

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Abbreviations

CTCs, circulating tumor cells; DCIS, ductal carcinoma *in situ*; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FGFs, fibroblast growth factors; HB-EGF, heparin-binding EGF-like growth factor; I-NETs, ileal neuroendocrine tumors; LOI, loss of imprinting; LPA, lysophosphatidic acid; MET, mesenchymal-epithelial transition; NC, neural crest; NETs, neutrophil extracellular traps; PDAC, pancreatic ductal adenocarcinoma; PMN, pre-metastatic niche; SYNJ2, synaptojanin 2; TGF, transforming growth factor.

References

- 1 Lambert, A.W., Pattabiraman, D.R. and Weinberg, R.A. (2017) Emerging biological principles of metastasis. *Cell* **168**, 670–691 <https://doi.org/10.1016/j.cell.2016.11.037>
- 2 Ganesh, K. and Massague, J. (2021) Targeting metastatic cancer. *Nat. Med.* **27**, 34–44 <https://doi.org/10.1038/s41591-020-01195-4>
- 3 Fidler, I.J. (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer* **3**, 453–458 <https://doi.org/10.1038/nrc1098>
- 4 Vogelstein, B., Papadopoulos, N., Velculescu, V.E., Zhou, S., Diaz, Jr, L.A. and Kinzler, K.W. (2013) Cancer genome landscapes. *Science* **339**, 1546–1558 <https://doi.org/10.1126/science.1235122>
- 5 Witsch, E., Sela, M. and Yarden, Y. (2010) Roles for growth factors in cancer progression. *Physiology (Bethesda)* **25**, 85–101 <https://doi.org/10.1152/physiol.00045.2009>
- 6 Virnig, B.A., Tuttle, T.M., Shamlivan, T. and Kane, R.L. (2010) Ductal carcinoma *in situ* of the breast: a systematic review of incidence, treatment, and outcomes. *J. Natl. Cancer Inst.* **102**, 170–178 <https://doi.org/10.1093/jnci/djp482>

- 7 Altevogt, P., Doberstein, K. and Fogel, M. (2016) L1CAM in human cancer. *Int. J. Cancer* **138**, 1565–1576 <https://doi.org/10.1002/ijc.29658>
- 8 Eming, S.A., Martin, P. and Tomic-Canic, M. (2014) Wound repair and regeneration: mechanisms, signaling, and translation. *Sci. Transl. Med.* **6**, 265sr6 <https://doi.org/10.1126/scitranslmed.3009337>
- 9 Cangkrama, M., Wietecha, M. and Werner, S. (2020) Wound repair, scar formation, and cancer: converging on activin. *Trends Mol. Med.* **26**, 1107–1117 <https://doi.org/10.1016/j.molmed.2020.07.009>
- 10 Finak, G., Bertos, N., Pepin, F., Sadekova, S., Souleimanova, M., Zhao, H. et al. (2008) Stromal gene expression predicts clinical outcome in breast cancer. *Nat. Med.* **14**, 518–527 <https://doi.org/10.1038/nm1764>
- 11 Keerthi, R., Dutta, A., Agarwal, S., Kani, V. and Khatua, A. (2018) Perineural invasion of oral squamous cell carcinoma: a new hurdle for surgeons. *J. Maxillofac. Oral Surg.* **17**, 59–63 <https://doi.org/10.1007/s12663-016-0943-1>
- 12 Liebig, C., Ayala, G., Wilks, J., Verstovsek, G., Liu, H., Agarwal, N. et al. (2009) Perineural invasion is an independent predictor of outcome in colorectal cancer. *J. Clin. Oncol.* **27**, 5131–5137 <https://doi.org/10.1200/JCO.2009.22.4949>
- 13 Lugassy, C., Kleinman, H.K., Vermeulen, P.B. and Barnhill, R.L. (2020) Angiotropism, pericytic mimicry and extravascular migratory metastasis: an embryogenesis-derived program of tumor spread. *Angiogenesis* **23**, 27–41 <https://doi.org/10.1007/s10456-019-09695-9>
- 14 Braun, A.D., Mengoni, M., Bonifati, S., Tuting, T. and Gaffal, E. (2020) Activated Hgf-Met signaling cooperates with oncogenic BRAF to drive primary cutaneous melanomas and angiotropic lung metastases in mice. *J. Invest. Dermatol.* **140**, 1410–1417.e2 <https://doi.org/10.1016/j.jid.2019.12.020>
- 15 Dupin, E., Calloni, G.W., Coelho-Aguiar, J.M. and Le Douarin, N.M. (2018) The issue of the multipotency of the neural crest cells. *Dev. Biol.* **444** Suppl 1, S47–S59 <https://doi.org/10.1016/j.ydbio.2018.03.024>
- 16 Kalluri, R. and Weinberg, R.A. (2009) The basics of epithelial–mesenchymal transition. *J. Clin. Invest.* **119**, 1420–1428 <https://doi.org/10.1172/JCI39104>
- 17 Gallik, K.L., Treffy, R.W., Nacke, L.M., Ahsan, K., Rocha, M., Green-Saxena, A. et al. (2017) Neural crest and cancer: Divergent travelers on similar paths. *Mech. Dev.* **148**, 89–99 <https://doi.org/10.1016/j.mod.2017.08.002>
- 18 Khalil, A.A., Iliina, O., Gritsenko, P.G., Bult, P., Span, P.N. and Friedl, P. (2017) Collective invasion in ductal and lobular breast cancer associates with distant metastasis. *Clin. Exp. Metastasis* **34**, 421–429 <https://doi.org/10.1007/s10585-017-9858-6>
- 19 Aceto, N., Bardia, A., Miyamoto, D.T., Donaldson, M.C., Wittner, B.S., Spencer, J.A. et al. (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**, 1110–1122 <https://doi.org/10.1016/j.cell.2014.07.013>
- 20 Varki, A. (2007) Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood* **110**, 1723–1729 <https://doi.org/10.1182/blood-2006-10-053736>
- 21 Nieswandt, B., Hafner, M., Echtenacher, B. and Mannel, D.N. (1999) Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res.* **59**, 1295–1300 PMID: 10096562
- 22 Boucharaba, A., Serre, C.M., Gres, S., Saulnier-Blache, J.S., Bordet, J.C., Guglielmi, J. et al. (2004) Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J. Clin. Invest.* **114**, 1714–1725 <https://doi.org/10.1172/JCI200422123>
- 23 David, M., Sahay, D., Mege, F., Descotes, F., Leblanc, R., Ribeiro, J. et al. (2014) Identification of heparin-binding EGF-like growth factor (HB-EGF) as a biomarker for lysophosphatidic acid receptor type 1 (LPA1) activation in human breast and prostate cancers. *PLoS One* **9**, e97771 <https://doi.org/10.1371/journal.pone.0097771>
- 24 Spiegel, A., Brooks, M.W., Houshyar, S., Reinhardt, F., Ardolino, M., Fessler, E. et al. (2016) Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. *Cancer Discov.* **6**, 630–649 <https://doi.org/10.1158/2159-8290.CD-15-1157>
- 25 Park, J., Wysocki, R.W., Amoozgar, Z., Maiorino, L., Fein, M.R., Jorns, J. et al. (2016) Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci. Transl. Med.* **8**, 361ra138 <https://doi.org/10.1126/scitranslmed.aag1711>
- 26 Weiss, L., Harlos, J.P. and Elkin, G. (1989) Mechanism of mechanical trauma to Ehrlich ascites tumor cells in vitro and its relationship to rapid intravascular death during metastasis. *Int. J. Cancer* **44**, 143–148 <https://doi.org/10.1002/ijc.2910440125>
- 27 Qian, B., Deng, Y., Im, J.H., Muschel, R.J., Zou, Y., Li, J. et al. (2009) A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS One* **4**, e6562 <https://doi.org/10.1371/journal.pone.0006562>
- 28 Furlow, P.W., Zhang, S., Soong, T.D., Halberg, N., Goodarzi, H., Mangrum, C. et al. (2015) Mechanosensitive pannexin-1 channels mediate microvascular metastatic cell survival. *Nat. Cell Biol.* **17**, 943–952 <https://doi.org/10.1038/ncb3194>
- 29 Strilic, B., Yang, L., Albarran-Juarez, J., Wachsmuth, L., Han, K., Muller, U.C. et al. (2016) Tumour-cell-induced endothelial cell necroptosis via death receptor 6 promotes metastasis. *Nature* **536**, 215–218 <https://doi.org/10.1038/nature19076>
- 30 Bos, P.D., Zhang, X.H., Nadal, C., Shu, W., Gomis, R.R., Nguyen, D.X. et al. (2009) Genes that mediate breast cancer metastasis to the brain. *Nature* **459**, 1005–1009 <https://doi.org/10.1038/nature08021>
- 31 Minn, A.J., Gupta, G.P., Padua, D., Bos, P., Nguyen, D.X., Nuyten, D. et al. (2007) Lung metastasis genes couple breast tumor size and metastatic spread. *Proc. Natl Acad. Sci. U.S.A.* **104**, 6740–6745 <https://doi.org/10.1073/pnas.0701138104>
- 32 Hood, J.L., San, R.S. and Wickline, S.A. (2011) Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer Res.* **71**, 3792–3801 <https://doi.org/10.1158/0008-5472.CAN-10-4455>
- 33 Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C. et al. (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438**, 820–827 <https://doi.org/10.1038/nature04186>
- 34 Li, R., Wen, A. and Lin, J. (2020) Pro-inflammatory cytokines in the formation of the pre-metastatic niche. *Cancers (Basel)* **12**, 3752 <https://doi.org/10.3390/cancers12123752>
- 35 Nair, N.U., Das, A., Rogkoti, V.-M., Fokkelman, M., Marcotte, R., de Jong, C.G. et al. (2019) Migration rather than proliferation transcriptomic signatures are strongly associated with breast cancer patient survival. *Sci. Rep.* **9**, 10989 <https://doi.org/10.1038/s41598-019-47440-w>
- 36 Lee, J.H., Zhao, X.M., Yoon, I., Lee, J.Y., Kwon, N.H., Wang, Y.Y. et al. (2016) Integrative analysis of mutational and transcriptional profiles reveals driver mutations of metastatic breast cancers. *Cell Discov.* **2**, 16025 <https://doi.org/10.1038/celldisc.2016.25>
- 37 Bersini, S., Lytle, N.K., Schulte, R., Huang, L., Wahl, G.M. and Hetzer, M.W. (2020) Nup93 regulates breast tumor growth by modulating cell proliferation and actin cytoskeleton remodeling. *Life Sci. Alliance* **3**, e201900623 <https://doi.org/10.26508/lsa.201900623>
- 38 Turajlic, S. and Swanton, C. (2016) Metastasis as an evolutionary process. *Science* **352**, 169–175 <https://doi.org/10.1126/science.aaf2784>

- 39 Russo, M., Crisafulli, G., Sogari, A., Reilly, N.M., Arena, S., Lamba, S. et al. (2019) Adaptive mutability of colorectal cancers in response to targeted therapies. *Science* **366**, 1473–1480 <https://doi.org/10.1126/science.aav4474>
- 40 Reiter, J.G., Makohon-Moore, A.P., Gerold, J.M., Heyde, A., Attiyeh, M.A., Kohutek, Z.A. et al. (2018) Minimal functional driver gene heterogeneity among untreated metastases. *Science* **361**, 1033–1037 <https://doi.org/10.1126/science.aat7171>
- 41 Sottoriva, A., Kang, H., Ma, Z., Graham, T.A., Salomon, M.P., Zhao, J. et al. (2015) A Big Bang model of human colorectal tumor growth. *Nat. Genet.* **47**, 209–216 <https://doi.org/10.1038/ng.3214>
- 42 Ocana, O.H., Corcoles, R., Fabra, A., Moreno-Bueno, G., Acloque, H., Vega, S. et al. (2012) Metastatic colonization requires the repression of the epithelial–mesenchymal transition inducer Prrx1. *Cancer Cell* **22**, 709–724 <https://doi.org/10.1016/j.ccr.2012.10.012>
- 43 Tsai, J.H., Donaher, J.L., Murphy, D.A., Chau, S. and Yang, J. (2012) Spatiotemporal regulation of epithelial–mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* **22**, 725–736 <https://doi.org/10.1016/j.ccr.2012.09.022>
- 44 Fischer, K.R. (2015) Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature* **527**, 472–476 <https://doi.org/10.1038/nature15748>
- 45 Zheng, X. (2015) Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature* **527**, 525–530 <https://doi.org/10.1038/nature16064>
- 46 Chen, Y., LeBleu, V.S., Carstens, J.L., Sugimoto, H., Zheng, X., Malasi, S. et al. (2018) Dual reporter genetic mouse models of pancreatic cancer identify an epithelial-to-mesenchymal transition-independent metastasis program. *EMBO Mol. Med.* **10**, e9085 <https://doi.org/10.15252/emmm.201809085>
- 47 Sinha, D., Saha, P., Samanta, A. and Bishayee, A. (2020) Emerging concepts of hybrid epithelial-to-mesenchymal transition in cancer progression. *Biomolecules* **10**, 1561 <https://doi.org/10.3390/biom10111561>
- 48 Aiello, N.M., Maddipati, R., Norgard, R.J., Balli, D., Li, J., Yuan, S. et al. (2018) EMT subtype influences epithelial plasticity and mode of cell migration. *Dev. Cell* **45**, 681–695.e4 <https://doi.org/10.1016/j.devcel.2018.05.027>
- 49 Reichert, M., Bakir, B., Moreira, L., Pitarresi, J.R., Feldmann, K., Simon, L. et al. (2018) Regulation of epithelial plasticity determines metastatic organotropism in pancreatic cancer. *Dev. Cell* **45**, 696–711.e8 <https://doi.org/10.1016/j.devcel.2018.05.025>
- 50 Pastushenko, I., Mauri, F., Song, Y., de Cock, F., Meeusen, B., Swedlund, B. et al. (2021) Fat1 deletion promotes hybrid EMT state, tumour stemness and metastasis. *Nature* **589**, 448–455 <https://doi.org/10.1038/s41586-020-03046-1>
- 51 Fumagalli, A., Oost, K.C., Kester, L., Morgner, J., Bornes, L., Bruens, L. et al. (2020) Plasticity of Lgr5-negative cancer cells drives metastasis in colorectal cancer. *Cell Stem Cell* **26**, 569–578.e7 <https://doi.org/10.1016/j.stem.2020.02.008>
- 52 Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W. et al. (2011) Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature* **480**, 118–122 <https://doi.org/10.1038/nature10598>
- 53 Aloysius, A., DasGupta, R. and Dhawan, J. (2018) The transcription factor Lef1 switches partners from beta-catenin to Smad3 during muscle stem cell quiescence. *Sci. Signal.* **11**, eaan3000 <https://doi.org/10.1126/scisignal.aan3000>
- 54 Biber, G., Ben-Shmuel, A., Sabag, B. and Barda-Saad, M. (2020) Actin regulators in cancer progression and metastases: from structure and function to cytoskeletal dynamics. *Int. Rev. Cell Mol. Biol.* **356**, 131–196 <https://doi.org/10.1016/bs.ircmb.2020.05.006>
- 55 Tavares, S., Vieira, A.F., Taubenberger, A.V., Araujo, M., Martins, N.P., Bras-Pereira, C. et al. (2017) Actin stress fiber organization promotes cell stiffening and proliferation of pre-invasive breast cancer cells. *Nat. Commun.* **8**, 15237 <https://doi.org/10.1038/ncomms15237>
- 56 Philippar, U., Rousson, E.T., Oser, M., Yamaguchi, H., Kim, H.D., Giampieri, S. et al. (2008) A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis. *Dev. Cell* **15**, 813–828 <https://doi.org/10.1016/j.devcel.2008.09.003>
- 57 Eckert, M.A., Lwin, T.M., Chang, A.T., Kim, J., Danis, E., Ohno-Machado, L. et al. (2011) Twist1-induced invadopodia formation promotes tumor metastasis. *Cancer Cell* **19**, 372–386 <https://doi.org/10.1016/j.ccr.2011.01.036>
- 58 Ben-Chetrit, N., Chetrit, D., Russell, R., Korner, C., Mancini, M., Abdul-Hai, A. et al. (2015) Synaptojanin 2 is a druggable mediator of metastasis and the gene is overexpressed and amplified in breast cancer. *Sci. Signal.* **8**, ra7 <https://doi.org/10.1126/scisignal.2005537>
- 59 Gewinner, C., Wang, Z.C., Richardson, A., Teruya-Feldstein, J., Etemadmoghadam, D., Bowtell, D. et al. (2009) Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell* **16**, 115–125 <https://doi.org/10.1016/j.ccr.2009.06.006>
- 60 Wrenn, E.D., Yamamoto, A., Moore, B.M., Huang, Y., McBirney, M., Thomas, A.J. et al. (2020) Regulation of collective metastasis by nonluminal signaling. *Cell* **183**, 395–410.e19 <https://doi.org/10.1016/j.cell.2020.08.045>
- 61 Durdu, S., Iskar, M., Revenu, C., Schieber, N., Kunze, A., Bork, P. et al. (2014) Luminal signalling links cell communication to tissue architecture during organogenesis. *Nature* **515**, 120–124 <https://doi.org/10.1038/nature13852>
- 62 Calissendorff, J., Maret, E., Sundin, A. and Falhammar, H. (2015) Ileal neuroendocrine tumors and heart: not only valvular consequences. *Endocrine* **48**, 743–755 <https://doi.org/10.1007/s12020-014-0446-0>
- 63 Hanahan, D. (1985) Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* **315**, 115–122 <https://doi.org/10.1038/315115a0>
- 64 Contractor, T., Clausen, R., Harris, G.R., Rosenfeld, J.A., Carpizo, D.R., Tang, L. et al. (2020) IGF2 drives formation of ileal neuroendocrine tumors in patients and mice. *Endocr. Relat. Cancer* **27**, 175–186 <https://doi.org/10.1530/ERC-19-0505>
- 65 Molnar, K., Meszaros, A., Fazakas, C., Kozma, M., Gyori, F., Reisz, Z. et al. (2020) Pericyte-secreted IGF2 promotes breast cancer brain metastasis formation. *Mol. Oncol.* **14**, 2040–2057 <https://doi.org/10.1002/1878-0261.12752>
- 66 Battle, E. and Massague, J. (2019) Transforming growth factor-beta signaling in immunity and cancer. *Immunity* **50**, 924–940 <https://doi.org/10.1016/j.immuni.2019.03.024>
- 67 Peinado, H., Zhang, H., Matei, I.R., Costa-Silva, B., Hoshino, A., Rodrigues, G. et al. (2017) Pre-metastatic niches: organ-specific homes for metastases. *Nat. Rev. Cancer* **17**, 302–317 <https://doi.org/10.1038/nrc.2017.6>
- 68 Costa-Silva, B., Aiello, N.M., Ocean, A.J., Singh, S., Zhang, H., Thakur, B.K. et al. (2015) Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat. Cell Biol.* **17**, 816–826 <https://doi.org/10.1038/ncb3169>
- 69 Wang, Z. (2017) ErbB receptors and cancer. *Methods Mol. Biol.* **1652**, 3–35 https://doi.org/10.1007/978-1-4939-7219-7_1
- 70 Singh, B., Carpenter, G. and Coffey, R.J. (2016) EGF receptor ligands: recent advances. *F1000Res* **5**, F1000 <https://doi.org/10.12688/f1000research.9025.1>

- 71 Zeng, X.Y., Xie, H., Yuan, J., Jiang, X.Y., Yong, J.H., Zeng, D. et al. (2019) M2-like tumor-associated macrophages-secreted EGF promotes epithelial ovarian cancer metastasis via activating EGFR-ERK signaling and suppressing lncRNA LIMT expression. *Cancer Biol. Ther.* **20**, 956–966 <https://doi.org/10.1080/15384047.2018.1564567>
- 72 Day, K.C., Lorenzatti Hiles, G., Kozminsky, M., Dawsey, S.J., Paul, A., Brodes, L.J. et al. (2017) HER2 and EGFR overexpression support metastatic progression of prostate cancer to bone. *Cancer Res.* **77**, 74–85 <https://doi.org/10.1158/0008-5472.CAN-16-1656>
- 73 Zhao, Y., Kaushik, N., Kang, J.H., Kaushik, N.K., Son, S.H., Uddin, N. et al. (2020) A feedback loop comprising EGF/TGF α sustains TFCP2-mediated breast cancer progression. *Cancer Res.* **80**, 2217–2229 <https://doi.org/10.1158/0008-5472.can-19-2908> PMID: 32193292
- 74 Lau, T.S., Chan, L.K., Wong, E.C., Hui, C.W., Sneddon, K., Cheung, T.H. et al. (2017) A loop of cancer-stroma-cancer interaction promotes peritoneal metastasis of ovarian cancer via TNF α -TGF α -EGFR. *Oncogene* **36**, 3576–3587 <https://doi.org/10.1038/nc.2016.509>
- 75 Zhuo, W. and Kang, Y. (2017) Lnc-ing ROR1-HER3 and Hippo signalling in metastasis. *Nat. Cell Biol.* **19**, 81–83 <https://doi.org/10.1038/ncb3467>
- 76 Haskins, J.W., Nguyen, D.X. and Stern, D.F. (2014) Neuregulin 1-activated ERBB4 interacts with YAP to induce Hippo pathway target genes and promote cell migration. *Sci. Signal.* **7**, ra116 <https://doi.org/10.1126/scisignal.2005770>
- 77 Berdiel-Acer, M., Maia, A., Hristova, Z., Borgoni, S., Vetter, M., Burmester, S. et al. (2021) Stromal NRG1 in luminal breast cancer defines pro-fibrotic and migratory cancer-associated fibroblasts. *Oncogene* **40**, 2651–2666 <https://doi.org/10.1038/s41388-021-01719-3>
- 78 Zhang, Z., Karthaus, W.R., Lee, Y.S., Gao, V.R., Wu, C., Russo, J.W. et al. (2020) Tumor microenvironment-derived NRG1 promotes antiandrogen resistance in prostate cancer. *Cancer Cell* **38**, 279–296.e9 <https://doi.org/10.1016/j.ccell.2020.06.005>
- 79 Wang, S., Zhang, Y., Wang, Y., Ye, P., Li, J., Li, H. et al. (2016) Amphiregulin confers regulatory T cell suppressive function and tumor invasion via the EGFR/GSK-3 β /Foxp3 axis. *J. Biol. Chem.* **291**, 21085–21095 <https://doi.org/10.1074/jbc.M116.717892>
- 80 Amara S, N.-A., Kuiken, H.J., Selfors, L.M., Butler, T., Leung, M.L., Leung, C.T. et al. (2020) Transient commensal clonal interactions can drive tumor metastasis. *Nat. Commun.* **11**, 5799 <https://doi.org/10.1038/s41467-020-19584-1>
- 81 Liu, S., Ye, D., Xu, D., Liao, Y., Zhang, L., Liu, L. et al. (2016) Autocrine epiregulin activates EGFR pathway for lung metastasis via EMT in salivary adenoid cystic carcinoma. *Oncotarget* **7**, 25251–25263 <https://doi.org/10.18632/oncotarget.7940>
- 82 Wang, Y., Jing, Y., Ding, L., Zhang, X., Song, Y., Chen, S. et al. (2019) Epiregulin reprograms cancer-associated fibroblasts and facilitates oral squamous cell carcinoma invasion via JAK2-STAT3 pathway. *J. Exp. Clin. Cancer Res.* **38**, 274 <https://doi.org/10.1186/s13046-019-1277-x>
- 83 Shen, Y., Ruan, L., Lian, C., Li, R., Tu, Z. and Liu, H. (2019) Discovery of HB-EGF binding peptides and their functional characterization in ovarian cancer cell lines. *Cell Death Discov.* **5**, 82 <https://doi.org/10.1038/s41420-019-0163-9>
- 84 Bellomo, C., Caja, L. and Moustakas, A. (2016) Transforming growth factor β as regulator of cancer stemness and metastasis. *Br. J. Cancer* **115**, 761–769 <https://doi.org/10.1038/bjc.2016.255>
- 85 David, C.J. and Massagué, J. (2018) Contextual determinants of TGF β action in development, immunity and cancer. *Nat. Rev. Mol. Cell Biol.* **19**, 419–435 <https://doi.org/10.1038/s41580-018-0007-0>
- 86 Pang, Y., Gara, S.K., Achyut, B.R., Li, Z., Yan, H.H., Day, C.P. et al. (2013) TGF- β signaling in myeloid cells is required for tumor metastasis. *Cancer Discov.* **3**, 936–951 <https://doi.org/10.1158/2159-8290.CD-12-0527>
- 87 Golan, T., Parikh, R., Jacob, E., Vaknine, H., Zemser-Werner, V., Hershkovitz, D. et al. (2019) Adipocytes sensitize melanoma cells to environmental TGF- β cues by repressing the expression of miR-211. *Sci. Signal.* **12**, eaav6847 <https://doi.org/10.1126/scisignal.aav6847>
- 88 Sundqvist, A., Vasilaki, E., Voytyuk, O., Bai, Y., Morikawa, M., Moustakas, A. et al. (2020) TGF β and EGF signaling orchestrates the AP-1- and p63 transcriptional regulation of breast cancer invasiveness. *Oncogene* **39**, 4436–4449 <https://doi.org/10.1038/s41388-020-1299-z>
- 89 Esposito, M., Fang, C., Cook, K.C., Park, N., Wei, Y., Spadazzi, C. et al. (2021) TGF- β -induced DACT1 biomolecular condensates repress Wnt signalling to promote bone metastasis. *Nat. Cell Biol.* **23**, 257–267 <https://doi.org/10.1038/s41556-021-00641-w>
- 90 Eckhardt, B.L., Cao, Y., Redfern, A.D., Chi, L.H., Burrows, A.D., Roslan, S. et al. (2020) Activation of canonical BMP4-SMAD7 signaling suppresses breast cancer metastasis. *Cancer Res.* **80**, 1304–1315 <https://doi.org/10.1158/0008-5472.CAN-19-0743>
- 91 Ceci, C., Atzori, M.G., Lacal, P.M. and Graziani, G. (2020) Role of VEGFs/VEGFR-1 signaling and its inhibition in modulating tumor invasion: experimental evidence in different metastatic cancer models. *Int. J. Mol. Sci.* **21**, 1388 <https://doi.org/10.3390/ijms21041388>
- 92 Apte, R.S., Chen, D.S. and Ferrara, N. (2019) VEGF in signaling and disease: beyond discovery and development. *Cell* **176**, 1248–1264 <https://doi.org/10.1016/j.cell.2019.01.021>
- 93 Li, R., Qi, Y., Jiang, M., Zhang, T., Wang, H., Wang, L. et al. (2019) Primary tumor-secreted VEGF induces vascular hyperpermeability in premetastatic lung via the occludin phosphorylation/ubiquitination pathway. *Mol. Carcinog.* **58**, 2316–2326 <https://doi.org/10.1002/mc.23120>
- 94 Yang, Y., Zhang, Y., Iwamoto, H., Hosaka, K., Seki, T., Andersson, P. et al. (2016) Discontinuation of anti-VEGF cancer therapy promotes metastasis through a liver revascularization mechanism. *Nat. Commun.* **7**, 12680 <https://doi.org/10.1038/ncomms12680>
- 95 English, W.R., Lunt, S.J., Fisher, M., Lefley, D.V., Dhingra, M., Lee, Y.C. et al. (2017) Differential expression of VEGFA isoforms regulates metastasis and response to anti-VEGFA therapy in sarcoma. *Cancer Res.* **77**, 2633–2646 <https://doi.org/10.1158/0008-5472.CAN-16-0255>
- 96 Yang, X., Zhang, Y., Hosaka, K., Andersson, P., Wang, J., Tholander, F. et al. (2015) VEGF-B promotes cancer metastasis through a VEGF-A-independent mechanism and serves as a marker of poor prognosis for cancer patients. *Proc. Natl Acad. Sci. U.S.A.* **112**, E2900–E2909 <https://doi.org/10.1073/pnas.1503500112>
- 97 Cao, R., Ji, H., Feng, N., Zhang, Y., Yang, X., Andersson, P. et al. (2012) Collaborative interplay between FGF-2 and VEGF-C promotes lymphangiogenesis and metastasis. *Proc. Natl Acad. Sci. U.S.A.* **109**, 15894–15899 <https://doi.org/10.1073/pnas.1208324109>
- 98 Kong, D., Zhou, H., Neelakantan, D., Hughes, C.J., Hsu, J.Y., Srinivasan, R.R. et al. (2021) VEGF-C mediates tumor growth and metastasis through promoting EMT-epithelial breast cancer cell crosstalk. *Oncogene* **40**, 964–979 <https://doi.org/10.1038/s41388-020-01539-x>
- 99 Chang, T.M., Chu, P.Y., Hung, W.C., Shan, Y.S., Lin, H.Y., Huang, K.W. et al. (2021) c-Myc promotes lymphatic metastasis of pancreatic neuroendocrine tumor through VEGFC upregulation. *Cancer Sci.* **112**, 243–253 <https://doi.org/10.1111/cas.14717>
- 100 Janiszewska, M., Tabassum, D.P., Castaño, Z., Cristea, S., Yamamoto, K.N., Kingston, N.L. et al. (2019) Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments. *Nat. Cell Biol.* **21**, 879–888 <https://doi.org/10.1038/s41556-019-0346-x>
- 101 Moschos, S.J. and Mantzoros, C.S. (2002) The role of the IGF system in cancer: from basic to clinical studies and clinical applications. *Oncology* **63**, 317–332 <https://doi.org/10.1159/000066230>
- 102 Baxter, R.C. (2014) IGF binding proteins in cancer: mechanistic and clinical insights. *Nat. Rev. Cancer* **14**, 329–341 <https://doi.org/10.1038/nrc3720>

- 103 Zhang, X.H., Jin, X., Malladi, S., Zou, Y., Wen, Y.H., Brogi, E. et al. (2013) Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* **154**, 1060–1073 <https://doi.org/10.1016/j.cell.2013.07.036>
- 104 Hirakawa, T., Yashiro, M., Doi, Y., Kinoshita, H., Morisaki, T., Fukuoka, T. et al. (2016) Pancreatic fibroblasts stimulate the motility of pancreatic cancer cells through IGF1/IGF1R signaling under hypoxia. *PLoS One* **11**, e0159912 <https://doi.org/10.1371/journal.pone.0159912>
- 105 Geleta, B., Park, K.C., Jansson, P.J., Sahni, S., Maleki, S., Xu, Z. et al. (2021) Breaking the cycle: Targeting of NDRG1 to inhibit bi-directional oncogenic cross-talk between pancreatic cancer and stroma. *FASEB J.* **35**, e21347 <https://doi.org/10.1096/fj.202002279R>
- 106 Zambelli, A., Biamenti, G. and Amato, A. (2021) HGF/c-Met signalling in the tumor microenvironment. *Adv. Exp. Med. Biol.* **1270**, 31–44 https://doi.org/10.1007/978-3-030-47189-7_2
- 107 Pothula, S.P., Xu, Z., Goldstein, D., Merrett, N., Pirola, R.C., Wilson, J.S. et al. (2017) Targeting the HGF/c-MET pathway: stromal remodelling in pancreatic cancer. *Oncotarget* **8**, 76722–76739 <https://doi.org/10.18632/oncotarget.20822>
- 108 Yin, J., Hu, W., Fu, W., Dai, L., Jiang, Z., Zhong, S. et al. (2019) HGF/MET regulated epithelial–mesenchymal transitions and metastasis by FOSL2 in non-small cell lung cancer. *Onco Targets Ther.* **12**, 9227–9237 <https://doi.org/10.2147/OTT.S217595>
- 109 Ding, X., Ji, J., Jiang, J., Cai, Q., Wang, C., Shi, M. et al. (2018) HGF-mediated crosstalk between cancer-associated fibroblasts and MET-unamplified gastric cancer cells activates coordinated tumorigenesis and metastasis. *Cell Death Dis.* **9**, 867 <https://doi.org/10.1038/s41419-018-0922-1>
- 110 Kitamura, T., Kato, Y., Brownlie, D., Soong, D.Y.H., Sugano, G., Kippen, N. et al. (2019) Mammary tumor cells with high metastatic potential are hypersensitive to macrophage-derived HGF. *Cancer Immunol. Res.* **7**, 2052–2064 <https://doi.org/10.1158/2326-6066.CCR-19-0234>
- 111 Xing, F., Liu, Y., Sharma, S., Wu, K., Chan, M.D., Lo, H.W. et al. (2016) Activation of the c-Met pathway mobilizes an inflammatory network in the brain microenvironment to promote brain metastasis of breast cancer. *Cancer Res.* **76**, 4970–4980 <https://doi.org/10.1158/0008-5472.CAN-15-3541>
- 112 Dianat-Moghadam, H. and Teimoori-Toolabi, L. (2019) Implications of fibroblast growth factors (FGFs) in cancer: from prognostic to therapeutic applications. *Curr. Drug Targets* **20**, 852–870 <https://doi.org/10.2174/1389450120666190112145409>
- 113 Ornitz, D.M. and Itoh, N. (2015) The fibroblast growth factor signaling pathway. *Wiley Interdiscip. Rev. Dev. Biol.* **4**, 215–266 <https://doi.org/10.1002/wdev.176>
- 114 Meng, X., Vander Ark, A., Daft, P., Woodford, E., Wang, J., Madaj, Z. et al. (2018) Loss of TGF- β signaling in osteoblasts increases basic-FGF and promotes prostate cancer bone metastasis. *Cancer Lett.* **418**, 109–118 <https://doi.org/10.1016/j.canlet.2018.01.018>
- 115 Wang, K., Ji, W., Yu, Y., Li, Z., Niu, X., Xia, W. et al. (2018) FGFR1-ERK1/2-SOX2 axis promotes cell proliferation, epithelial–mesenchymal transition, and metastasis in FGFR1-amplified lung cancer. *Oncogene* **37**, 5340–5354 <https://doi.org/10.1038/s41388-018-0311-3>
- 116 Roussigné, M., Wei, L., Tsingos, E., Kuchling, F., Alkobtawi, M., Tsalavouta, M. et al. (2018) Left/right asymmetric collective migration of parapineal cells is mediated by focal FGF signaling activity in leading cells. *Proc. Natl Acad. Sci. U.S.A.* **115**, E9812–E9821 <https://doi.org/10.1073/pnas.1812016115>
- 117 Östman, A. (2017) PDGF receptors in tumor stroma: biological effects and associations with prognosis and response to treatment. *Adv. Drug Deliv. Rev.* **121**, 117–123 <https://doi.org/10.1016/j.addr.2017.09.022>
- 118 Kazlauskas, A. (2017) PDGFs and their receptors. *Gene* **614**, 1–7 <https://doi.org/10.1016/j.gene.2017.03.003>
- 119 Wyss, C.B., Duffey, N., Peyvandi, S., Barras, D., Martinez Usatorre, A., Coquoz, O. et al. (2021) Gain of HIF1 activity and loss of miRNA let-7d promote breast cancer metastasis to the brain via the PDGF/PDGFR axis. *Cancer Res.* **81**, 594–605 <https://doi.org/10.1158/0008-5472.CAN-19-3560>
- 120 Yang, Y., Andersson, P., Hosaka, K., Zhang, Y., Cao, R., Iwamoto, H. et al. (2016) The PDGF-BB-SOX7 axis-modulated IL-33 in pericytes and stromal cells promotes metastasis through tumour-associated macrophages. *Nat. Commun.* **7**, 11385 <https://doi.org/10.1038/ncomms11385>
- 121 Yoon, H., Tang, C.M., Banerjee, S., Yebra, M., Noh, S., Burgoyne, A.M. et al. (2021) Cancer-associated fibroblast secretion of PDGFC promotes gastrointestinal stromal tumor growth and metastasis. *Oncogene* **40**, 1957–1973 <https://doi.org/10.1038/s41388-021-01685-w>
- 122 Hosaka, K., Yang, Y., Seki, T., Fischer, C., Dubey, O., Fredlund, E. et al. (2016) Pericyte-fibroblast transition promotes tumor growth and metastasis. *Proc. Natl Acad. Sci. U.S.A.* **113**, E5618–E5627 <https://doi.org/10.1073/pnas.1608384113>
- 123 Lopez-Yrigoyen, M., Cassetta, L. and Pollard, J.W. (2020) Macrophage targeting in cancer. *Ann. NY Acad. Sci.* <https://doi.org/10.1111/nyas.14377> PMID: 32445205. online ahead of print
- 124 da Silva, S.D., Marchi, F.A., Su, J., Yang, L., Valverde, L., Hier, J. et al. (2021) Co-overexpression of TWIST1-CSF1 is a common event in metastatic oral cancer and drives biologically aggressive phenotype. *Cancers (Basel)* **13**, 153 <https://doi.org/10.3390/cancers13010153>
- 125 Lu, C.S., Shiau, A.L., Su, B.H., Hsu, T.S., Wang, C.T., Su, Y.C. et al. (2020) Oct4 promotes M2 macrophage polarization through upregulation of macrophage colony-stimulating factor in lung cancer. *J. Hematol. Oncol.* **13**, 62 <https://doi.org/10.1186/s13045-020-00887-1>
- 126 Sánchez-González, I., Bobien, A., Molnar, C., Schmid, S., Strotbek, M., Boerries, M. et al. (2020) miR-149 SUPPRESSES BREAST CANCER METASTASIS BY BLOCKING PARACRINE INTERACTIONS WITH MACROPHAGES. *Cancer Res.* **80**, 1330–1341 <https://doi.org/10.1158/0008-5472.CAN-19-1934>
- 127 Nusse, R. and Clevers, H. (2017) Wnt/ β -catenin signaling, disease, and emerging therapeutic modalities. *Cell* **169**, 985–999 <https://doi.org/10.1016/j.cell.2017.05.016>
- 128 Wang, Y., Singhal, U., Qiao, Y., Kasputis, T., Chung, J.S., Zhao, H. et al. (2020) Wnt signaling drives prostate cancer bone metastatic tropism and invasion. *Transl. Oncol.* **13**, 100747 <https://doi.org/10.1016/j.tranon.2020.100747>
- 129 Moon, J.H., Lee, S.H. and Lim, Y.C. (2021) Wnt/ β -catenin/Slug pathway contributes to tumor invasion and lymph node metastasis in head and neck squamous cell carcinoma. *Clin. Exp. Metastasis* **8**, 163–174 <https://doi.org/10.1007/s10585-021-10081-3>
- 130 Jiang, S., Li, Q., Liu, Y., Zhang, H., Wang, Q., Chen, Y. et al. (2021) Activation of WNT7b autocrine eases metastasis of colorectal cancer via epithelial to mesenchymal transition and predicts poor prognosis. *BMC Cancer* **21**, 180 <https://doi.org/10.1186/s12885-021-07898-2>
- 131 Karagiannis, G.S., Rivera-Sanchez, L., Duran, C.L., Oktay, M.H. and Condeelis, J.S. (2020) Emerging roles of Cxcl12/Cxcr4 signaling axis in breast cancer metastasis. *J. Immunol.* **204**, 90.4 PMID: 18814042
- 132 Sun, X., Cheng, G., Hao, M., Zheng, J., Zhou, X., Zhang, J. et al. (2010) CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression. *Cancer Metastasis Rev.* **29**, 709–722 <https://doi.org/10.1007/s10555-010-9256-x>
- 133 Subramanian, B., Sridharan, S., Howard, M., Basuroy, T., M C Tilley, A., de la Serna, I. et al. (2020) Role of the CXCR4-LASP1 axis in the stabilization of snail1 in triple-negative breast cancer. *Cancers (Basel)* **12**, 2372 <https://doi.org/10.3390/cancers12092372>
- 134 Luo, N., Chen, D.D., Liu, L., Li, L. and Cheng, Z.P. (2019) CXCL12 promotes human ovarian cancer cell invasion through suppressing ARHGAP10 expression. *Biochem. Biophys. Res. Commun.* **518**, 416–422 <https://doi.org/10.1016/j.bbrc.2019.07.098>

- 135 Tulotta, C., Stefanescu, C., Chen, Q., Torraca, V., Meijer, A.H. and Snaar-Jagalska, B.E. (2019) CXCR4 signaling regulates metastatic onset by controlling neutrophil motility and response to malignant cells. *Sci. Rep.* **9**, 2399 <https://doi.org/10.1038/s41598-019-38643-2>
- 136 Yang, F., Takagaki, Y., Yoshitomi, Y., Ikeda, T., Li, J., Kitada, M. et al. (2019) Inhibition of dipeptidyl peptidase-4 accelerates epithelial–mesenchymal transition and breast cancer metastasis via the CXCL12/CXCR4/mTOR axis. *Cancer Res.* **79**, 735–746 <https://doi.org/10.1158/0008-5472.CAN-18-0620>
- 137 Ahrwar, D.K., Nasser, M.W., Ouseph, M.M., Elbaz, M., Cuitiño, M.C., Kladney, R.D. et al. (2018) Fibroblast-derived CXCL12 promotes breast cancer metastasis by facilitating tumor cell intravasation. *Oncogene* **37**, 4428–4442 <https://doi.org/10.1038/s41388-018-0263-7>
- 138 Bernat-Peguera, A., Simón-Extremera, P., da Silva-Diz, V., López de Munain, M., Díaz-Gil, L., Penin, R.M. et al. (2019) PDGFR-induced autocrine SDF-1 signaling in cancer cells promotes metastasis in advanced skin carcinoma. *Oncogene* **38**, 5021–5037 <https://doi.org/10.1038/s41388-019-0773-y>
- 139 Redmer, T. (2018) Deciphering mechanisms of brain metastasis in melanoma - the gist of the matter. *Mol. Cancer* **17**, 106 <https://doi.org/10.1186/s12943-018-0854-5>
- 140 Yang, Y., Ma, Y., Gao, H., Peng, T., Shi, H., Tang, Y. et al. (2021) A novel HDGF-ALCAM axis promotes the metastasis of Ewing sarcoma via regulating the GTPases signaling pathway. *Oncogene* **40**, 731–745 <https://doi.org/10.1038/s41388-020-01485-8>