

## Growth Factors and their receptors in cancer metastases

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## 1. ABSTRACT

Metastatic, rather than primary tumours are responsible for ninety percent cancer deaths. Despite significant advances in the understanding of molecular and cellular mechanisms in tumour metastases, there are limitations in preventive treatment of metastatic tumours. Much evidence arising from laboratory and clinical studies suggests that growth factors and their receptors are implicated in cancer metastases development. We review the origin and production of growth factors and their receptors in all stages of cancer metastases including epithelial-mesenchymal transition, cancer cell invasion and migration, survival within the circulation, seeding at distant organs and metastatic tumour angiogenesis. The functions of growth factors and their receptors are also discussed. This review presents the efforts made in understanding this challenge to aid in the development of new treatment strategies for cancer metastases.

## 2. INTRODUCTION

Cancer metastasis is a phenomenon during which tumour cells escape from primary cancer, transfer and re-grow in a new organ. Metastatic tumours are very common in the late stages of cancer and account for ninety percent human cancer deaths (1). To develop new metastatic tumour, cancer cells need to endure and survive a series of complex processes including epithelial-mesenchymal transition, dislodgement from the primary tumour, transportation through the circulation, extravasation and establishment at a distant site. Because of its clinical importance, significant advances in the understanding of molecular and cellular mechanisms of tumour invasion and metastasis have been made and summarised a decade ago (2). Much evidence arising from laboratory and clinical studies suggests that growth factors and their receptors appear to play critical roles in all stage of cancer metastases development. This article will review the pathogenesis of

cancer metastasis; the origin and production of growth factors and their receptors in all stages of cancer metastasis including epithelial-mesenchymal transition, cancer cell invasion and migration, survival within circulation, seeding at distant organs and metastatic tumour angiogenesis. The function of these growth factors and their receptors in cancer metastasis development will also be discussed.

### 3. THE PATHOGENESIS OF CANCER METASTASIS

The pathogenesis of cancer metastasis is a spread of cancer cells from primary tumours to distant organs. The metastatic process can be divided into epithelial-mesenchymal transition (EMT), cancer cell invasion and migration, cancer cell survival in the circulation, cell seeding at distant organs and cancer metastasis angiogenesis.

#### 3.1. Epithelial-mesenchymal transition (EMT)

To detach from the primary tumour and invade into the surrounding tissue, tumour cells have to break down cell-cell contacts, remodel cell-matrix adhesion sites, and transit from an epithelial phenotype to a mesenchymal phenotype, this transition is called epithelial-mesenchymal transition (EMT). EMT is an early stage of cell migration and invasion which occurs during tumour progression and is characterised by the disruption of intercellular junctions and the replacement of apical-basolateral polarity with front-to-back polarity. EMT transforms cancer cells from an epithelial morphology to a migratory and invasive phenotype which is critical for the metastasis of many carcinomas (3). Loss of E-cadherin function is a key step in the EMT process (4). E-cadherin is a major component of epithelial adherens junctions and mediates intercellular adhesion in epithelial cancers. Loss of E-cadherin function not only leads to a mechanical disruption of adherens junctions, but also liberates proteins from the cytoplasmic cell adhesion complex which exert ambivalent functions depending on their subcellular localization (4). Following a series of molecular processes the cellular static actin structures are reorganized, a pliable membrane protrusion forms and membrane ruffling occurs. After loss of epithelial E-cadherin, cancer cells start to express mesenchymal N-cadherin which has a specific affinity for mesenchymal cells. This cadherin switch leads to a drastic change in the adhesive properties of a cell, as it loses its affinity for its epithelial neighbours and gains affinity for mesenchymal cells, such as fibroblasts or vascular endothelial cells (4).

#### 3.2. Cancer cell invasion and migration

The ability of a neoplastic cell to undergo migration and invasion allows it to change position within the tumour tissue and to enter lymphatic and blood vessels for dissemination into the circulation. The principles of cell migration were initially studied in non-neoplastic cells (5). Additional research on tumour cells show the mechanisms of migration are preserved in neoplastic cells (6). To migrate, the cell body modifies its shape and stiffness to interact with the surrounding tissue structure which results from a continuous cycle of interdependent steps. The protein-protein interactions and signalling events that

underlie shape changes regulate these steps, and the extracellular matrix (ECM) provides the substrate for the advancing cell body. Novel imaging techniques (7), together with re-evaluation of histopathological patterns in tumours have provided a detailed view of cellular and molecular migration dynamics which shows cancer-cell migration is regulated by integrins (adhesion molecules) and matrix-degrading enzymes through the cell-cell adhesion and communication (6). The cells disseminated from primary tumours can have amoeboid movement as individual cells or be a mesenchymal-type movement as cell clusters. Cancer therapeutics designed to target these adhesion receptors or proteases have not yet been shown to be effective in clinical trials due to the fact that cancer cell migration mechanisms can be altered allowing it to retain its invasive properties.

#### 3.3. Cancer cell survival in circulation

A study using a green fluorescent protein technique viewed living tumour cells during their intravasation and found a large number of cells fragment when interacting with blood vessels (8). The fact that a tumour releases many thousands of cancer cells into the circulation every day but only a few of them successfully form metastases (9) indicates the processes of cancer metastasis are rather inefficient, and some of them are rate limiting. The low survival rate in the circulation has been linked with the higher level of apoptosis in circulating cancer cells (10). Examination of the relationship between a cancer cell apoptotic property and its metastatic potential has revealed that apoptosis is a safeguard to avoid metastasis (11). When cancer cells are impaired in their apoptotic properties they will have survival advantages with a greater chance to result in metastasis. For example, when cancer cells lost the ability to undergo apoptosis due to the *p53* (a pro-apoptotic protein) gene mutation, cancer patients had a higher level of lymphatic and vascular invasion and a worse outcome with a shorter survival time compared to patients who did not have mutations in the *p53* genes (12). Therefore apoptosis has been regarded as a multistep barrier to metastasis (10) and the mechanisms by which metastatic cancer cell resist apoptosis have become targets to treat metastatic cancers (13).

#### 3.4. Cell seeding at distant organs

After successfully surviving from apoptotic challenges in the circulation, metastatic cells can be transferred to a new organ where they adhere to the vessel wall and extravasate into the organ parenchyma. An investigation into the efficiency of the metastatic process using intravital video-microscopy has revealed that only 1% of injected cancer cells developed into micro-metastases and most cells undergo apoptosis (14). Clinical observations and studies in the *in vivo* model have revealed that certain tumour types tend to metastasize to specific organs. For example, prostate cancer cell favourably metastasize to bone and lymph nodes; the most common sites that breast cancer spreads to are bone, lymph nodes, and the lung; Seventy five percent colorectal cancer metastases occur in the liver. Interestingly some sites such as muscle are rarely if ever the site of metastasis. Even in

the same organ metastases can develop in diverse anatomical sites depending on the cancer cell types. For example, injection of two different types of melanoma tumour cells into the internal carotid artery of mice developed brain melanoma metastases at entirely different sites; one type of melanoma cells developed lesions only in the brain parenchyma, whereas another types of cells produced metastases only in meningeal areas (15). This suggests that the different sites of tumour growth within one organ might be based on the interaction between the metastatic cells and the host organ environment- probable related to specific binding to stroma cells or responses to local growth factors.

### 3.5. Cancer metastasis angiogenesis

Tumour angiogenesis is a process of new blood vessel formation. After colonization in a new organ site, metastatic cells can develop into a micro-tumour of 1-2 mm diameter without angiogenesis. However, since decreased diffusion of nutrients and oxygen into tumours leads to necrosis (16), angiogenesis is required for the tumour to grow beyond this size. Hypoxia and ischemia are the main stimuli for angiogenesis initiation. In response to hypoxia and ischemia the endothelial cells secrete matrix metalloproteases (MMP), which digest the blood-vessel walls to enable them to escape and migrate toward the site of the angiogenic stimuli. Protein fragments produced by the digestion of the blood-vessel walls intensify the proliferative and migratory activity of endothelial cells, which form a capillary tube by altering the arrangement of their adherence-membrane proteins. Through the process of anastomosis, the capillaries derived from the arterioles and the venules join together and form a continuous blood flow. Angiogenesis is a complex and highly regulated process with a large number of pro-angiogenic growth factors being involved. Another interesting phenomenon during metastasis development is tumour dormancy. Tumour dormancy is a concept referring to the failure of some small metastasis (less than 1 to 2 mm diameter) to increase further in size because of an absence of angiogenesis (17). A study which investigated dormant lung metastases under angiogenesis suppression in mice showed that metastases remain dormant due to the balance between cell proliferation and apoptosis within the tumour (18). Other studies also found that growth of tumour cells immediately after extravasation or after a period of dormancy is regulated by a combination of effectors, such as growth factors, the extracellular matrix and the microenvironment in which they are located (14, 19, 20). The tumour dormancy presents an important clinical challenge as dormant cancer cells have a low proliferative index and would be unaffected by therapies directed against dividing cells, but they have the potential to be activated at a later time and commence growth. These cells would be analogous to time bombs hidden within the tissue, and it will be important for us to learn how to control these dormant cancer cells.

## 4. GROWTH FACTORS AND THEIR RECEPTOR IN CANCER METASTATIC PATHOGENESIS

It has been well established that all primary tumour contain a diverse population of stroma cells in addition to cancerous cells. These stroma cells are activated

and recruited to form a local microenvironment which initially exerts an inhibitory effect on malignant cells (19). During tumour development cancerous cells avoid inhibitory signals and turn these cells to a statue which result in the tumour's inappropriate growth, invasion and ultimately metastasis. Although the molecular mechanism of these pro-metastatic functions of the microenvironment remains poorly elucidated, many growth factors and their receptors have been implicated in metastasis development (Figure 1).

### 4.1. Growth factors in epithelial-mesenchymal transition (EMT)

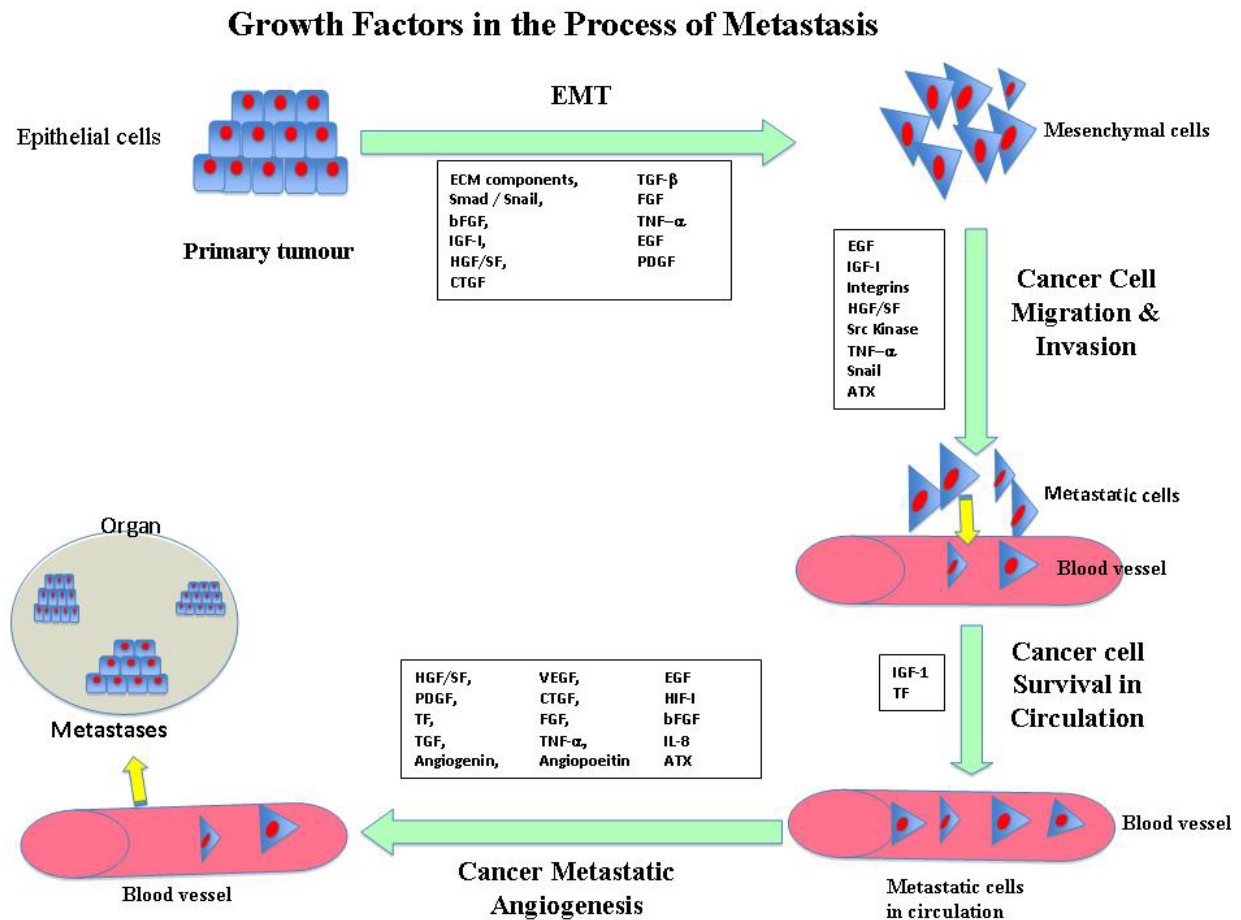
Interaction of extracellular signals including components of the extracellular matrix (ECM) and soluble growth factors play a major role in the EMT process. The involved growth factors include transforming growth factor- $\beta$  (TGF- $\beta$ ), Smad, fibroblast growth factor (FGF) families, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), insulin-like growth factors (IGFs), epidermal growth factor (EGF) and hepatocyte growth factor (HGF). As IGFs, EGF and HGF are also involved in the late stage of metastasis; they will not be discussed in this section, but will be included in the subsequent discussions.

#### 4.1.1. Transforming Growth Factor- $\beta$ (TGF- $\beta$ )

TGF- $\beta$  is a member of a growth factor family that regulates cellular proliferation, differentiation, apoptosis and extracellular matrix formation (21-23). TGF- $\beta$  usually serves as a tumour suppressor in the normal tissues by inhibiting cell proliferation and inducing apoptosis (24), but it promotes tumour progression and invasion if the tumour cells overcome its cytostatic and apoptotic effects (25). In fact TGF- $\beta$  is one of the most potent inducers of EMT both in cultured cells and animal models (26). It initiates carcinogenic EMT in different systems *in vitro* and *in vivo* (27), inhibits the growth of epithelial cells and promotes the growth of mesenchymal cells (28). TGF- $\beta$  signals through three cell surface receptors: the type I (T $\beta$ RI), type II (T $\beta$ RII) and type III (T $\beta$ RIII) receptors. T $\beta$ RIII can bind all TGF- $\beta$  isoforms and presents them to T $\beta$ RII (29). After binding with ligand, T $\beta$ RII recruits and phosphorylates T $\beta$ RI to activate its kinase activity. T $\beta$ RI then phosphorylates and activates Smad2/3, which bind to Smad4, and the complex accumulates in the nucleus and interacts with other transcription factors to regulate the expression of a multitude of target genes (29). In addition to being involved in the regulation of EMT, TGF- $\beta$  itself is also regulated by other growth factors, such as connective tissue growth factor (CTGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF). These growth factors affect EMT through mediation of TGF- $\beta$  action or regulation of TGF- $\beta$  expression (30).

#### 4.1.2. SMADs

SMADs are a group of protein which mediate downstream signaling of TGF- $\beta$ . A recent study which investigated the role of SMADs in the EMT process found that Smad signalling is necessary for TGF- $\beta$  induced



**Figure 1.** Growth factors in different stages of cancer metastatic pathogenesis Primary tumour cells endure and survive a series of complex processes including epithelial-mesenchymal transition, dislodgement from the primary tumour, transportation through the circulation, extravasation and establishment at a distant site. Many growth factors are implicated in all stages of these processes.

epigenetic silencing in breast cancer cells (31). This suggested that TGF $\beta$ -TGF $\beta$ R-Smad signalling axis tightly regulates EMT through a positive mechanism. But in some cases TGF $\beta$ -TGF $\beta$ R-Smad signalling axis regulated EMT through a negative feedback mechanism (30). For example Smad7 binds E3-ubiquitin ligases of the smurf family which cause ubiquitination and proteasomal degradation of the TGF- $\beta$  receptors (32) and prevents the phosphorylation of Smad2 and Smad3, providing a negative feedback loop mechanism to shut off the TGF $\beta$  (33). These contrary observations suggest that TGF $\beta$ -TGF $\beta$ R-Smad signalling pathways are regulated through both positive and negative mechanisms which tightly control EMT in cancer cells.

#### 4.1.3. Snail

The snail is a group of zinc-finger transcription factors which have central role in embryonic morphogenesis as it is essential for the formation of

mesoderm and neural crest that requires large scale cell movement. Snail has a fundamental role in EMT by suppressing E-cadherin expression, inhibiting the expression of other epithelial molecules and inducing the expression of genes associated with a mesenchymal and invasive phenotype (34). The fact that Snail is only expressed in both epithelial and endothelial cells of invasive breast cancer but not in normal breast tissue (35) and that its expression is associated with metastasis and poor prognosis (36), suggests that Snail is involved with tumour metastasis development. Snail is a highly unstable protein and TNF- $\alpha$  was found to be a major factor to stabilize it (37). It also has also been shown that TNF- $\alpha$  greatly enhances the migration and invasion of tumour cells by inducing the EMT program via its Snail stabilization actions (37). Knockdown of Snail expression not only inhibits TNF- $\alpha$ -induced cancer cell migration and invasion *in vitro*, but also suppresses metastasis *in vivo*. This

suggests that Snail could be a novel target for cancer metastasis prevention and treatment.

### 4.2. Growth factors in initial steps of metastasis

#### 4.2.1. Autocrine Motility Factor (AMF)

Liotta et al (38) isolated a cell motility stimulating factor from serum free conditioned medium of melanoma cells and named it as autocrine motility factor (AMF). AMF was subsequently identified as a glycolytic enzyme termed glucose-6-phosphate isomerase (GPI) (39), therefore it was re-named as AMF/PGI (40). PGI is a housekeeping cytosolic enzyme that plays a key role in both glycolysis and gluconeogenesis pathways. AMF/PGI appears to be a multifunctional protein that displays cytokine properties, eliciting mitogenic, motogenic, and differentiation activities, and has been implicated in tumour progression and metastasis (38, 41, 42). It has been found in a wide spectrum of malignancies acting extracellularly through its receptor (AMFR E3), but its glycolysis activity is not required for its cytokine function (43). This represents an example of a complex ligand-receptor system linking intracellular and extracellular events including metabolism and cytokine signalling. The cytokine activity of AMF/PGI and its receptor has been reviewed recently by Fairbank et al (40).

#### 4.2.2. Autotaxin (ATX)

Autotaxin (ATX) was extracted from human melanoma cells (44) as a motility-stimulating protein. Subsequently it was found in a variety of tumour cells, including those from prostate and breast carcinomas, melanomas, neuroblastomas and renal-cell carcinoma (45, 46). ATX has been shown to promote tumour aggressiveness, metastasis and angiogenesis in mouse model (47), its expression is associated with the glioblastoma (48), follicular lymphoma (49) and uveal melanoma (50) cell motility. Its presence might turn cancer cells into very aggressive, metastasis-induced tumour with poor prognoses (51). The motility capacity of ATX was tentatively linked to its phosphate hydrolysing activity with key endobiotic metabolites in cells (52). Sequence analysis revealed that ATX gene shares significant homologous sequences with a family of cell surface phosphodiesterases (53), it therefore has been regarded as a phospholipase D (52). Its role in cancer metastasis process has been recently reviewed in full (52).

#### 4.2.3. Epidermal Growth Factor (EGF)

EGF and its receptor (EGFR) have been associated with tumour cell invasion and metastasis initiation (54). Dysregulation of EGFR signalling, including receptor over expression and/or activation has been shown to be a significant effector in the progression of human cancers including neoplasms of the brain, lung, breast, ovary, prostate, and pancreas (55). A recent study investigated the relationship between EGFR and the adhesion molecule-integrin in human pancreatic carcinoma cells and demonstrated that the crosstalk between EGFR signalling and integrin in the cancer cell membrane is implicated in carcinoma cell invasion and metastasis (56). Integrins are a family of adhesion proteins that regulate cell migration. The fact that EGF stimulated integrins-mediated

carcinoma cell migration on vitronectin suggests that EGFR regulates cancer cell migration through the adhesion proteins, the integrins. EGFR inhibitors, such as erlotinib, provide clinical benefit in patients with advanced non-small cell lung cancer metastasis (57) which suggests a critical role for EGF and its receptor in the initial steps of cancer metastasis. The mechanism of EGF activation of adhesion proteins in cancer cell remains to be elucidated. Some studies indicate EGF induces tumour cell invasion and metastasis through de-phosphorylation and down-regulation of focal adhesion kinase (54), while other studies suggest EGFR activates the Src family of kinases (SFK) (58). The fact that activated Src kinase is involved in the rearrangement of the actin cytoskeleton, cell-matrix interactions, and cell-cell adhesion processes that promote cell invasion (56) suggests a role for Src activity in tumour metastasis development.

#### 4.2.4. Hepatocyte Growth Factor (HGF)

HGF is the most potent mitogen for mature hepatocytes in primary culture and acts as a trigger for liver regeneration after partial hepatectomy and liver injury (59). It is also implicated in the metastatic spread of tumours as a scatter factor and has been proposed as a strong and independent predictor of recurrence in human breast cancer (60, 61). Its receptor (cMET) is over-expressed in most human cancers (62). Colon cancer cell invasion and motility potential is significantly increased following incubation with HGF (63). This suggests that HGF plays an important role in cancer metastasis initiation. Membrane ruffling is an early event in cell movement; HGF induces rapid membrane ruffling, formation of microspikes and increased cell motility in colon cancer cells (64) indicating HGF enhances cell motility through induction of cell membrane ruffling. An investigation into the tyrosine phosphorylation and translocation of ruffling proteins in colon cancer cells has found that HGF stimulates the function of the ruffling protein (ezrin) which initiates cancer cell membrane ruffling and other early signals for cancer cells to move and invade (65). The mechanisms which trigger cancer cell membrane ruffling are largely unestablished. A further study indicated that cytosolic free  $\text{Ca}^{2+}$  may be involved in the mechanism (64). In addition to acting as a cancer cell motility and invasion stimulator, HGF also enhances cancer angiogenesis by increasing Vascular Endothelial Growth Factor (VEGF) promoter activity (66) and inducing hypoxia inducible factor-1 (HIF-1) expression (67). The pivotal role and the comprehensive function of HGF and its receptor (cMET) in cancer metastasis initiation and development has been summarised by Jiang et al (68).

#### 4.2.5. Insulin-like growth factor system (IGFs)

The insulin-like growth factor system consists of two ligands (IGF-I and -II), two main receptors (IGF-IR and IGFIIR), six different IGF binding proteins (IGFBP1-6) and four IGFBP related peptides (IGFBP Rp1-4). The IGF ligands have a short life-span unless they are bound to a binding protein which transports them in the circulation and delivers them to specific tissues. Components of the IGF system are found throughout the body in various fluids and tissues (69, 70). IGFs act on a variety of mammalian

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cells in an endocrine, paracrine and autocrine manner (71) to regulate cell proliferation, apoptosis, transformation and differentiation (72, 73). They influence the growth of normal tissue as well as that of several cancers. Converging data from clinical and laboratory studies clearly indicate that IGF-I is implicated in cancer cell migration and invasion (74-76). IGF-I receptor (IGF-IR) expression is correlated with colorectal cancer venous invasion and liver metastasis, and has been proposed as a predictor of liver metastasis from colorectal cancer (77). Blockade of the paracrine action of IGF-I can suppress liver metastases from colorectal cancer (78). It has been established that IGF-IR and the integrins interact together to form a complex at the colon cell-cell contact sites, whilst addition of IGF-I to this complex causes integrin redistribution within the cell-cell contact site and is associated with an increase in the migration of colorectal cancer cells (79).

### 4.2.6. Tumour Necrosis Factor- $\alpha$ (TNF- $\alpha$ )

TNF- $\alpha$  is synthesised as a transmembrane protein with a molecular mass of 26 kDa which is cleaved by TNF- $\alpha$  converting enzyme (TACE) and secreted as a soluble peptide factor. TNF- $\alpha$  is produced by a variety of cells such as activated macrophages, fibroblasts, Kupffer cells, smooth muscle cells, breast and colorectal carcinoma cells. It plays a major role in establishing the link between inflammation and cancer, and contributes to the development of tissue architecture necessary for tumour growth and metastasis (34). TNF- $\alpha$  mediates its effect through two different receptors: TNF- $\alpha$  receptor I (TNF-R1) and TNF- $\alpha$  receptor II (TNF-R2) (80). TNF-R1 is expressed in all cell types while TNF-R2 is expressed only in endothelial and immune cells. TNF- $\alpha$  usually promotes metastatic cell migration through its effects on tumour cells, stroma and inflammatory cells within the tumour microenvironment (34). For example, an investigation into the role of TNF- $\alpha$  on lung fibrosarcoma metastases in a mice model found that TNF- $\alpha$  confers an invasive and transformed phenotype to tumour cells, and increases lung metastases (81). It promotes breast cancer cell migration through up-regulation of LOX (82) and enhances the invasiveness of mammary epithelial cells through induction of MMPs or  $\alpha 2\beta 1$  integrin (83). It has been proven that the TNF- $\alpha$ , both exogenous and macrophage-produced, enhances the invasive property of cancer cells through the Snail-dependent mechanisms (84, 85). The migration and invasiveness promoting activities suggest that inhibition of TNF- $\alpha$  could be an effective strategy for cancer therapy. Some clinical trials using TNF- $\alpha$  antagonists such as anti-TNF- $\alpha$  antibody or TNF- $\alpha$  synthesis inhibitors to block TNF- $\alpha$  action in various tumours and have shown the promising effects (86, 87).

### 4.3. Growth factors and the circulation of cancer cell

It is known that low survival rate of cancer cells in circulation is associated with a higher level of apoptosis in circulating cancer cells (10), but there are few studies which investigate the cancer cell survival mechanism in circulation. So far two growth factors have been found to be associated with cancer cell survival in the circulation. These are IGF-I and tissue factor (TF).

#### 4.3.1. IGF-I

Sachdev et al (88) stably transfected breast cancer cells with a truncated IGF-I receptor and made the cancer cells loss affinity to IGF-I. When these cells were injected into the mammary fat pads of mice, it was found these cancer cells were able to develop to a primary tumour, but failed to form metastases, while the same cancer cells with the wild type of IGF-I receptor can develop both a primary and metastases (88). These findings show that the normal IGFIR function is required for cancer cell survival and development of metastasis. A recent study (89) injected the same IGF-I receptor defect cancer cells or anti-IGF-I receptor antibody to inhibit IGF-I receptor in mice and found that the disruption of IGF-I receptor resulted in a significantly decreased number of circulating tumour cells in the blood stream suggesting IGF-I signaling is necessary for cancer cell survival in the circulation. IGF-I has been established as a strong anti-apoptotic growth factor (13). Interfering with IGF-I signaling with an IGF-IR antagonist peptide has been shown to markedly induce cancer cell apoptosis indicating IGF-I signaling is a survival factor for cancer cells (90).

#### 4.3.2. Tissue factor (TF)

A study using a three-dimensional visualization system to observe platelet-tumour cell interactions in vessels found that coagulation is involved in tumour cell spreading within vessels and that anticoagulant treatment can inhibit cancer cell spreading on the vessels (91). Further studies found that tissue factor (TF) and TF-mediated thrombin generation appears to be the mechanism linking the enhancement of coagulation on metastasis (92). It seems that tumour cell-induced TF binds to two main coagulation factors (factor VIIa and X). This mediates the formation of tumour cell-associated microthrombi which forms a platelet aggregate around metastatic cancer cells in vessels and protects them from being destroyed by nature killer cells (92). The fact that treatment with a variety of anticoagulants decrease metastases in experimental models and in patients (93) further implicate the role of coagulation on cancer cell survival in the systemic circulation.

### 4.4. Growth factor and cancer metastatic angiogenesis

Using intravital videomicroscopy to examine injected cancer cells in mouse liver microvasculature, Luzzi et al (14) found that individual cell failure to initiate growth and micrometastases failure to continue develop into macroscopic tumours are two major barriers for the extravasated tumour cells to develop to metastases in new organs. Of these cells only 1% undergo proliferation and develop into a macroscopic tumour, 4% proceed to apoptosis, while 95% become dormant (14). It is imaginable that this large pool of dormant cells has the potential to be activated at some later time, which would be consistent with clinical evidence that human malignancies can recur years after apparently successful treatment of a primary tumour (94). Angiogenesis is a key player for small tumours (<1 to 2 mm diameter) to develop into a macroscopic tumour. The combination of factors inherent to individual cells and the microenvironment such as growth factors and extracellular matrix may also play a major role in this process. Many growth factors, including

hypoxia-inducible Factor (HIF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor (TGF), hepatocyte growth factor (HGF), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), angiogenin, interleukin (IL)-8 and the angiopoietins, have been implicated in this process. TGF, HGF and TNF- $\alpha$  have been included in previous discussion; therefore they will not be discussed in this section.

### 4.4.1. Hypoxia-inducible Factor (HIF)

HIF is a sequence-specific DNA-binding protein that can promote or repress the transcription of a broad range of genes which are involved in maintaining biological homeostasis. HIF is mostly non-functional in oxygenated cells under normal conditions and has a central role in oxygen sensing. A fast proliferation of metastatic cells in new organs may create distance between tumour cells and the blood vessels leading to a hypoxic microenvironment, and activate HIF (95). HIF, in turn, initiates adaptive survival processes which maintain metabolic equilibrium, pH homeostasis (96) and autophagy (97). This reinforces metastatic tumour growth (98). The development of pharmacological approaches that inhibit HIF or its target-gene products may provide therapeutic benefit in cancer patients (99).

### 4.4.2. Vascular Endothelial Growth Factor (VEGF)

VEGF is the key regulator in tumour angiogenesis and expressed in all solid tumours studied (100). Its expression has been regarded as a risk factor for metastases from colon (101) and breast cancer (102). VEGF belongs to a family of angiogenic factors and consists of several isoforms termed as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and PlGF (103). Among these factors VEGF-A plays an essential role in angiogenesis which binds to two tyrosine kinase receptors, named VEGFR-1 and VEGFR-2. VEGF-A have six isoforms with different lengths of amino acid residues resultant from alternative splicing (103). VEGF-A is involved in every stage of vascular development due to its potential of inducing endothelial cell proliferation and survival. The capability of VEGF in promotion of vascular endothelial cell (EC) proliferation has been well documented in *in vitro* and *in vivo* models (104), its pro-survival effect on EC has been suggested by a study in which the ablation of VEGF has significantly increased apoptosis of EC (105). VEGF is also a vascular permeability factor which enhances vascular leakage and permeability (104). Induction of vascular permeability is an essential early step in angiogenesis which results in leakage of plasma proteins, including fibrinogen and other clotting proteins. The clotting system is rapidly activated by tissue factors and results in the deposition of extravascular fibrin in tumour stroma; the fibrin can transform the anti-angiogenic stroma into a provisional stroma that is strongly pro-angiogenic (106). Consistent with a role in the regulation of vascular permeability, VEGF also induces endothelial fenestration in some vascular beds (104).

### 4.4.3. Fibroblast growth factor (FGF)

FGF family consists of at least 22 members; most of them are single-chain peptides and display a high affinity to heparin and heparin sulfate (107). FGFs stimulate a variety of cellular functions by binding FGF-

receptors in the presence of heparin proteoglycans. The FGF-receptor family is composed of seven members (107); all of them are single-chain receptor tyrosine kinases. After binding by ligands, the FGF-receptor becomes activated and gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation, and matrix dissolution, thus initiating a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells. Of all 22 members in the FGF family, FGF-1 (acidic FGF) is the broadest-acting member which can bind to all seven FGF-receptor subtypes, and promote diverse cell type growth. In contrast to from other growth factors, FGF-1 stimulates the proliferation and differentiation of all cell types necessary for building an arterial vessel, including endothelial and smooth muscle cells (108). FGF-2 (basic FGF) is another important member of FGF family which stimulates endothelial cell proliferation and enhances endothelial cells forming the tube-like structures (108).

### 4.4.4. Angiogenin (ANG)

ANG was initially isolated as a tumour-cell secreted polypeptide growth factor but was subsequently found to be a normal constituent of human plasma. Its up-regulation has been associated with progression and metastasis in prostate (109), breast (110) and colorectal cancer (111) and correlates with a low response to treatment and a poor survival rate (112). ANG can bind to actin on the surface of endothelial cells where it is endocytosed and translocated to the nucleus. Once in the nucleus it promotes endothelial invasiveness and enhances blood vessel formation. ANG also acts directly on cancer cells; for example, it can act as an effective substrate for cancer cell adhesion during metastasis (113), and blockade of ANG nucleus translocation in mice tumour model can reduce cancer cell proliferation and decrease tumour angiogenesis (114). In addition to its actions in tumour metastasis, ANG may also function as a tRNA-specific ribonuclease which can abolish protein synthesis by specifically hydrolyzing cellular tRNAs (115).

### 4.4.5. Interleukin-8 (IL-8)

IL-8 was initially identified as a pro-inflammatory cytokine. Substantial evidence exists that IL-8 is a critical factor in angiogenesis in a multitude of human tumors (116) and is apparently involved in angiogenesis in distant metastasis from colorectal cancer (117). The fact that the expression level of IL-8 is much higher in metastatic breast tumour cells than in non-metastatic breast tumour cells suggests a potential role for IL-8 in the metastatic phenotype of breast carcinomas (118). IL-8 binds to two G-protein coupled receptors (GPCR), IL-8 receptor A (CXCR1) and IL-8 receptor B (CXCR2). IL-8 receptors, unlike IL-8, are constitutively expressed in both mesenchymal and epithelial cells (119). IL-8 has mitogenic and chemotactic effect on endothelial cells.

### 4.4.6. Angiopoietins (Angs)

Angs are a group of protein growth factors including four isoforms, termed as Ang1, Ang2, Ang3 and Ang4, and are able to promote angiogenesis in both

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**Table 1.** The origins and actions of growth factors in different stage of metastasis

Growth Factor	Symbol	Receptor	Origin	Action	Stage	References
Activin A (inhibinBA+ inhibin BA)	INHBA	ACVR1, ACVR1A, ACVR1C, ACVR2A ACVR2B	CRC cells, others	proliferation, differentiation, apoptosis	primary, metastatic	(123)
Amphiregulin	AREG	EGFR	CRC cells, others	unclear; growth effects depend on cell type	primary, metastatic	(124)
Autocrine Motility Factor	AMF/PGI	AMFR E3	melanoma cells	mitogenic, motogenic, differentiation	primary, metastatic	(38, 40)
Bone morphogenic protein 2	BMP2	BMPRI1A, BMPRI1B, BMPRI2	CRC cells, others	inhibits growth, pro-apoptotic, differentiation	primary, metastatic	(125, 126)
Bone morphogenic protein 3	BMP3	BMPRI1A, BMPRI1B, BMPRI2	CRC cells, others	negative growth regulator	primary	(127)
Bone morphogenic protein 4	BMP4	BMPRI1A, BMPRI1B, BMPRI2	CRC cells, others	migration and invasion	intermediate, metastatic	(128)
Bone morphogenic protein 7	BMP7	BMPRI1A, BMPRI1B, BMPRI2	CRC cells, others	invasion	primary, intermediate, metastatic	(129)
Colony stimulating factor 2 (granulocyte macrophage)	CSF2 (GM-CSF)	CSF2RA, CSF2RB	Hematopoietic cells	proliferation	primary	(130, 131)
Colony stimulating factor 3 (granulocyte)	CSF3 (G-CSF)	CSF3R	Hematopoietic cells , CRC cells	unknown; overexpressed in CRC	metastatic	(132)
Colony stimulating factor 1 (macrophage)	CSF1 (M- CSF)	CSF1R	Hematopoietic cells	angiogenesis, tumor progression	primary, metastatic	(133)
Epidermal growth factor	EGF	EGFR	Numerous cell types	proliferation, differentiation, survival	primary, metastatic	(134)
Epiregulin	EREG	EGFR	CRC cells, others	unclear; growth effects depend on cell type	primary, metastatic	(135, 136)
Fibroblast growth factor 1	FGF1	FGFR1	Fibroblasts, CRC cells	angiogenesis, proliferation	primary, metastatic	(137)
Fibroblast growth factor 2	FGF2	FGFR2	Fibroblasts, CRC cells	angiogenesis, proliferation	primary	(138)
Fibroblast growth factor 7	FGF7 (KGF)	FGFR2iib	Fibroblasts, CRC cells	angiogenesis, proliferation, differentiation, migration, adhesion	primary	(139)
Fibroblast growth factor 10	FGF10	FGFR2iib	Mesenchymal cells	growth	primary	(140)
Growth differentiation factor 11	GDF11	ACVR1B, ACVR1C, TGFBRI1	CRC cells, others	unknown; overexpression correlates with metastasis	primary	(141)
Heparin-binding EGF- like growth factor	HB-EGF	EGFR	CRC cells, others	growth	primary	(134)
Insulin-like growth factor 1	IGF-I	IGF1R	Liver, CRC cells, others	proliferation, anti-apoptotic, migration	primary, metastatic	(142)
Insulin-like growth factor 2	IGF-II	IGF2R	Liver, CRC cells, others	proliferation, migration	primary, metastatic	(143)
Interleukin 2	IL-2	IL2RA, IL2RB, IL2RG	Hematopoietic cells	growth, survival	primary, metastatic	(144)
Interleukin 3	IL-3	IL3RA, IL3RB	Hematopoietic cells	proliferation, differentiation	primary	(145)
Interleukin 4	IL-4	IL4RA, IL2RG	Hematopoietic cells	anti-apoptotic, invasion, proliferation	primary, metastatic	(146)
Interleukin 5	IL-5	IL5RA, IL3RB	Hematopoietic cells	tumor progression	intermediate, metastatic	(147)
Interleukin 6	IL-6	IL6RA, IL6B	Hematopoietic cells	growth, invasion	primary, metastatic	(148)
Interleukin 7	IL-7	IL7RA, IL2RG	Hematopoietic cells	growth, tumor progression	Primary, metastatic	(149)
Interleukin 8	IL-8	IL8RA, IL8RB	Hematopoietic cells	tumor progression	primary, metastatic	(150)
Interleukin 10	IL-10	IL10RA, IL10RB	Hematopoietic cells	tumor progression	primary, metastatic	(151)
Interleukin 11	IL-11	IL11RA	Bone marrow stroma cells	Bone metastasis Primary osteosarcoma	primary, metastatic	(152)
Interleukin 12	IL-12	IL12RA, IL12RB1	Hematopoietic cells	anti-metastatic	intermediate, metastatic	(153)
Interleukin 13	IL-13	IL13RA1, IL4RA	Hematopoietic cells	tumor progression, growth	primary, metastatic	(154)
Interleukin 15	IL-15	IL15RA	Intraepithelial	Anti-apoptotic	primary	(155)



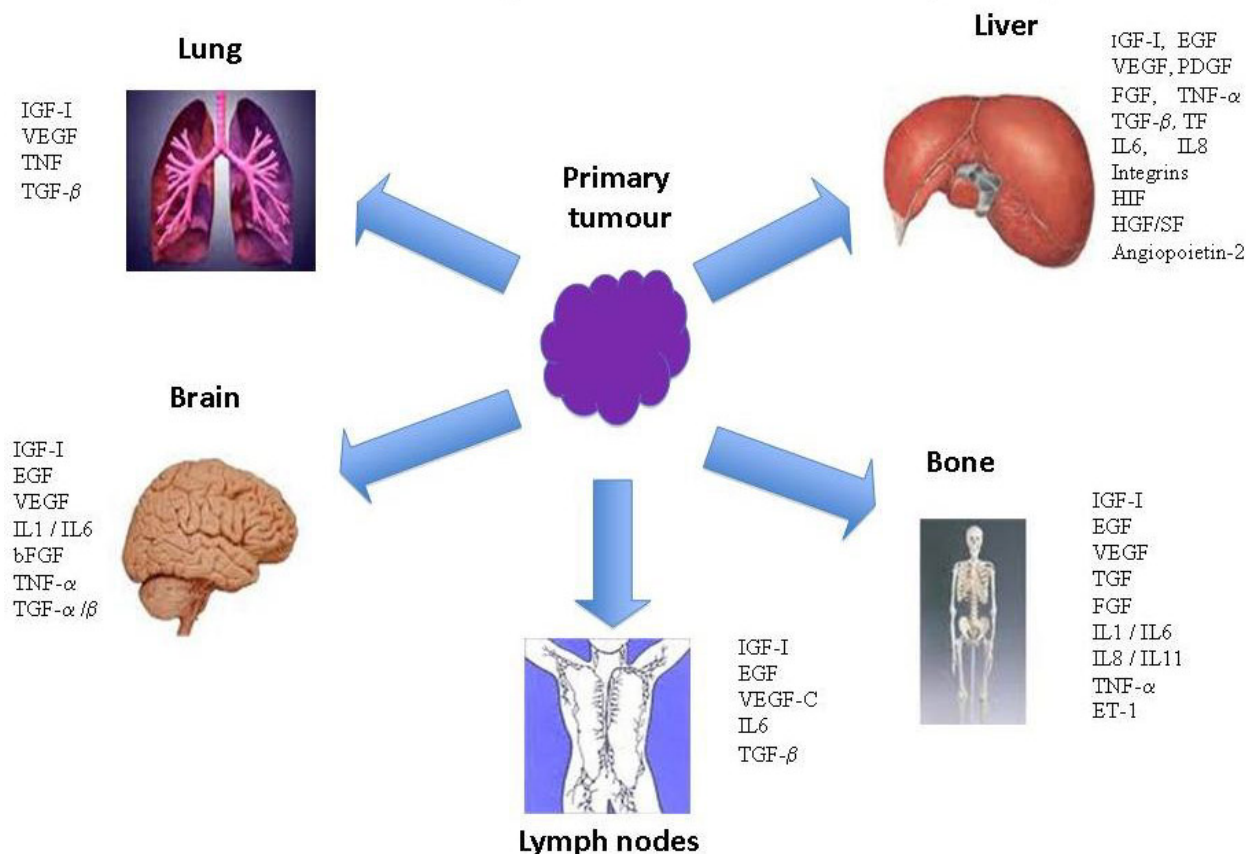
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			lymphocytes			
Interleukin 16	IL-16	CD4	Hematopoietic cells	Pro-inflammatory, additional roles unknown	primary	(156)
Interleukin 17	IL-17	IL17RA, IL17RB	Epithelium, endothelium, other	Pro-inflammatory, angiogenesis, tumor progression	primary, metastatic	(157)
Inhibin BA	INHBA	Unknown	CRC cells, others	unclear; tumor expression increases with disease progression	primary, metastatic	(158)
INF-alpha	INFA1	INFAR1, INFAR2	Hematopoietic cells	angiogenesis	primary, intermediate	(159)
INF-beta	INFB1	INFAR1, INFAR2	Hematopoietic cells	anti-growth, pro-apoptotic	primary, metastatic	(160)
INF-gamma	INFG	INFGR	Hematopoietic cells	anti-growth, apoptotic	primary, metastatic	(161)
Kit ligand	KITLG, (SCF)	c-KIT	Hematopoietic cells	proliferation, invasion	primary, metastatic	(145)
Leptin	LEP	LEPR	Adipocytes	proliferation	primary	(162)
Lymphotoxin alpha	LTA (TNF-beta)	LTBR	CRC cells, others	pro-apoptotic	primary	(163)
Nicotinamide phosphoribosyl-transferase	NAMPT (PBEF)	INSR	Beta cells, adipocytes, others	Unknown; overexpressed in CRC	primary	(164)
Platelet-derived growth factor alpha	PDGFA	PDGFRA, PDGFRB	Hematopoietic cells, others	proliferation, differentiation, angiogenesis, migration	primary, metastatic	(165)
Platelet-derived growth factor alpha/beta	PDGF-AB	PDGFRA, PDGFRB	Hematopoietic cells, others	proliferation, differentiation, angiogenesis, migration	primary	(166)
Platelet-derived growth factor beta	PDGFB	PDGFRA, PDGFRB	CRC cells, others	proliferation, differentiation, angiogenesis, migration	primary, intermediate	(167)
Resistin	RETN	TLR4	Adipocytes, macrophages, epithelial cells,	inflammation, angiogenesis, invasion, progression	primary, metastatic	(168)
Tumor necrosis factor	TNF	TNFR	CRC cells, others	pro-apoptotic	primary	(169)
Transforming growth factor, alpha	TGFA	EGFR	Macrophages, neurons, keratinocytes,	proliferation, tumor progression	primary, metastatic	(170)
Transforming growth factor, beta 1	TGFB1	TGFB1	Leukocytes	proliferation, differentiation, apoptosis, tumor progression	primary, intermediate	(171)
Transforming growth factor, beta 2	TGFB2	TGFB2R	Fibroblasts	proliferation, invasion	metastatic	(172)
Vascular endothelial growth factor	VEGF	FLT1	CRC cells, others	angiogenesis	intermediate, metastatic	(173)
Wingless-type MMTV integration site family member 1	WNT1	FZD1	CRC cells, others	proliferation, differentiation, migration	primary, intermediate	(174)
Wingless-type MMTV integration site family member 2	WNT2	FZD4	CRC cells, others	proliferation, migration, contact-independent growth	primary	(175)
Wingless-type MMTV integration site family member 4	WNT4	FZD6	CRC cells, others	proliferation, migration, contact-independent growth	primary	(176)
Wingless-type MMTV integration site family member 5A	WNT5A	FZD5	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate	(177)
Wingless-type MMTV integration site family member 6	WNT6	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate	(178)
Wingless-type MMTV integration site family member 7A, 7B	WNT7A, WNT7B	FZD5, FZD10, FZD9	CRC cells, others	proliferation, migration, contact-independent growth	primary	(179)
Wingless-type MMTV integration site family member 8B	WNT8B	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate	(180)
Wingless-type MMTV integration site family member 10A	WNT10A	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate	(178)
Wingless-type MMTV integration site family member 11	WNT11	FZ7	CRC cells, others	proliferation, migration, cytoskeletal rearrangement, contact-independent growth	primary, intermediate	(181)

primary and metastatic tumours. These growth factors can bind to two receptors Tie-1 and Tie-2, which are mainly expressed in endothelial cells. Observations have shown the conflicting activity of Angs toward endothelial cells. Ang1 has been shown to promote endothelial cell proliferation and therefore enhanced tumour angiogenesis (120).

Conversely another study demonstrated that Angs lacked mitogenic activity towards endothelial cells but affects distinct aspects of vascular development (121). These disparate effects may be due to the different distribution of Tie1 and Tie2 in endothelial cells as these two receptors possibly interact with each other and prevent each others

## Growth Factors and Development of Metastases in Major Organs



**Figure 2.** Growth factors and development of metastases in major organs The most common metastatic sites from solid tumours are the lungs, liver, brain, bone and lymph nodes. Many growth factors are involved in the development of metastatic tumour in these organs.

activation (121). Nevertheless, Angs have been demonstrated to be necessary for the formation of mature blood vessels by mouse knock out studies (122).

### 5. PERSPECTIVE

Carcinoma cells can spread from their primary location to other parts of the body. The most common metastatic sites from solid tumours are the lungs, liver, brain and bone. Once cancer becomes metastatic it cannot be effectively treated by surgery or chemotherapy and is responsible for ninety percent cancer patient deaths. Some metastatic carcinoma cells fail to develop into metastases due to the balance between cell proliferation and apoptosis within a new organ and instead become dormant. Dormant cancer cells have a low proliferative index, but hold the potential to be activated at a later time and commence growth. This presents an important clinical challenge and therefore it is important for us to understand the molecular and cellular mechanisms governing tumour metastases. Many growth factors and their receptors are implicated in cancer metastasis development which has been summarised here (Figure 2, Table 1). We hope this will add to the efforts in understanding this challenging

problem and help in the development of new treatment strategies for cancer metastases.

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