review

Interplay of distinct growth factors during epithelial–mesenchymal transition of cancer progenitor cells and molecular targeting as novel cancer therapies

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In this review, we describe the critical functions assumed by the interplay of epidermal growth factor, hedgehog, Wnt/β-catenin, tumor growth factor-β and integrin signaling cascades in tumorigenic and migrating cancer progenitor cells and activated stromal cells during carcinogenesis. These growth factors provide an important role for the sustained growth and survival of tumorigenic cancer progenitor cells and their progeny by up-regulating numerous mitotic and antiapoptotic signaling cascades. Furthermore, these potent morphogens may cooperate for inducing the molecular events associated with the epithelial–mesenchymal program in cancer cells including the alterations in epithelial cell shape and motility through the dissociation of intercellular adherens junctions. Of therapeutic interest, new strategies for the development of more effective clinical treatments against the locally aggressive and invasive cancers based on the molecular targeting of deregulated signaling elements in tumorigenic and migrating cancer cells and their local microenvironment are also described.

Key words: cancer progenitor cells, epithelial–mesenchymal transition, invasion, metastasis, stromal microenvironment, targeting therapies

introduction

Numerous recent lines of evidence have revealed that the accumulation of genetic and/or epigenetic alterations in the multipotent adult stem cells and/or their early progeny may contribute to their oncogenic transformation into tumorigenic and migrating cancer progenitor cells during cancer progression [1–16]. More specifically, the highly tumorigenic cancer progenitor cells expressing the stem cell-like markers such as Sca-1, CD133, CD44, Oct-3/4, c-KIT and/or xenobiotic efflux pumps associated with multidrug resistance have been isolated from acute myeloid leukemia (AML) as well as in primary and/or secondary neoplasms from patients with skin, brain, breast, prostate and ovarian cancers and in tumors established from cancer cell lines [1, 5, 8, 11, 12, 16–26]. These cancer progenitor cells also designated as cancer stem cells or cancer-initiating cells, and which are able to give rise to more differentiated cancer cell types in vitro and in vivo, appear to play a critical role for the tumor formation, progression and metastasis at distant sites (Figure 1). In this matter, the up-regulated expression of diverse tumorigenic target gene products in cancer progenitor cells and their progeny induced through the activation of distinct developmental signaling pathways such as epidermal growth factor (EGF)/epidermal growth factor receptor (EGFR), hedgehog, Wnt/β-catenin, Notch, tumor growth factor-β (TGF-β) and/or integrin cascades may contribute to their sustained growth and survival (Figures 1 and 2) [12, 16, 27–34]. Furthermore, these growth factor networks may cooperatively participate in the events leading to the disruption of the cell–cell adhesion contacts, and tumor cell dissociation and migration during the epithelial–mesenchymal transition (EMT) program (Figure 1) [16, 27–34]. Importantly, the enhanced expression of a subset of mesenchymal genes during the EMT program in certain tumorigenic cancer progenitor cells in primary neoplasm may notably contribute to their acquisition of a migratory and invasive behavior, and thereby leads to the progression to metastatic and recurrent disease states [16, 34].

In addition, the cancer progression is also accompanied by an extensive remodeling of the extracellular matrix (ECM) components and changes in the gene expression pattern in the activated fibroblasts as well as infiltrating circulating endothelial cells and immune cells such as macrophages in reactive stroma occurring during the EMT process [16, 35, 36]. Particularly, the tumor stromal cells secrete a variety of growth
Figure 1. Proposed model showing the possible oncogenic changes occurring during the malignant transformation of adult stem cells into tumorigenic and migrating cancer progenitor cells during the cancer progression. This model involves that the oncogenic transformation of adult stem cells may lead to the generation of tumorigenic cancer progenitor cells which can acquire a specific malignant phenotype dependent on their local microenvironment and the oncogenic cascades activated in them during the cancer progression. More specifically, certain poorly- or moderately-differentiated and tumorigenic cancer progenitor cells could acquire a migratory phenotype during epithelial–mesenchymal transition (EMT) program while other tumorigenic cancer progenitor cell types could not undertake the EMT transition. Hence, the activation of specific oncogenic cascades in cancer progenitor cells during cancer initiation and progression could result in different highly or weakly invasive cancer subtypes characterized by a poorly- to moderately differentiated state. In addition, this model also implicates that the acquisition of a migratory phenotype by certain poorly- or moderately-differentiated and tumorigenic cancer progenitor cells in primary neoplasm could represent a determinant factor contributing to their invasion and metastasis to distant tissues/organs.
factors and cytokines such as EGF, insulin-like growth factor (IGF), hepatocyte growth factor (HGF) and TGF-β as well as matrix metalloproteinases (MMPs) and urokinase plasminogen activator (uPA) that may contribute in a paracrine manner to events associated with the malignant transformation of cancer cells during the EMT process (Figure 1) [9, 16, 27, 28, 35, 37]. Hence, the integration of these distinct internal and external signals may promote the ability of tumorigenic and migrating cancer progenitor cells to evade from the primary cancer site and spread to distant locations where they can establish their novel homing. The persistence of these tumorigenic and migrating cancer progenitor cells in locally invasive and metastatic cancers to current clinical treatments may be responsible for the disease relapse. In this matter, we describe the molecular events that are often associated with the EMT program during the early and late stages of cancer progression with a particular emphasis on the critical functions assumed by growth factors, cytokines and integrins. Of particular interest, novel therapeutic strategies which are based on the molecular targeting of the oncogenic signaling elements that are often deregulated in tumorigenic and migrating cancer progenitor cells and host cells during the transition from premalignant lesions into aggressive and invasive forms are also reported.

### Localized cancer

The EMT phenomenon, which occurs during embryonic development and tissue injuries, is also reactivated during the progression from numerous cancers such as skin, prostate, mammary, hepatic, gastrointestinal (GI), pancreatic and colorectal carcinomas into locally aggressive and invasive forms [9, 29, 30, 34, 35]. The EMT process implicates complex changes in cancer cells and their local microenvironment which may lead to a decreased cell–cell adhesion, cell detachment and enhanced cancer cell–ECM component interactions in reactive stroma (Figure 1). These molecular events occurring during EMT program are accompanied by the morphogenetic changes in polarized cancer epithelial cells concomitant with a deregulation of cell–cell adhesion junctions that result to a loss of the epithelial phenotype and acquisition of mesenchymal properties conferring an enhanced motility and invasive ability to cancer cells. This is generally associated with a sustained activation...
of diverse oncogenic cascades in the epithelial cells during the progression from premalignant lesions into locally invasive cancers.

**early-stage EMT program**

During the early stages of carcinogenesis, a disorganization of epithelial cell–cell junctional complexes including adherens-, tight-, gap- and/or desmosomal junctions may occur during the EMT process [13, 16, 27–30, 33, 35, 36, 38–42]. This may result in the disruption of intercellular adhesion and cell–cell dissociation, and thereby lead to the detachment of cancer cells from the tumor mass. For instance, in the adheren junctions, the extracellular domain of cadherins such as E-cadherin participates in the homophilic interactions on adjacent cells while its intracellular region interacts with the catenins which are linked to the actin cytoskeleton. The activation of receptor tyrosine kinases (RTKs) such as the HGF (Met) receptor, EGFR, platelet-derived growth factor receptor (PDGFR) and Src tyrosine kinase may disrupt the adherens junctions including catenin–E-cadherin complexes by tyrosine phosphorylation of cadherin components [43]. This may result in the disruption of cell polarity and locomotion. Moreover, the EMT process is also accompanied by the down-regulation of the expression levels and/or redistribution of other junctional component types that may acquire an intracellular localization [43]. Hence, these molecular events ultimately lead to a loss of polarity and changes in the structural shape and behavior of cancer cells due to a disorganization of cell–cell interactions and the actin cytoskeleton. Moreover, this is also accompanied by the acquisition of a migratory fibroblastoid phenotype by cancer cells as indicated by the expression of mesenchymal markers such as vimentin and a switch from E-cadherin to N-cadherin expression [30, 33].

In addition, the EMT program is also accompanied by the activation of mechanisms that are involved in the cell detachment-induced apoptotic death (anoikis). In fact, a positive selection occurs during which certain cancer cell subpopulations and adjacent normal epithelial cells can trigger apoptotic death, while other tumor cells, including the cancer progenitor cells or their early progeny possessing the oncogenic phenotype advantages, can survive [28, 29, 34]. In this matter, the normal basal epithelial cells in the primary neoplasms, including prostate and breast carcinomas, are gradually destroyed during the transition from low-, intermediate- and high-grade intraepithelial neoplasms into well-established invasive cancers (Figure 1). Thus, it appears that the insults occurring in these normal basal cells may trigger their apoptotic death [44]. More particularly, it has been reported that an increase in the expression level of plasminogen activator inhibitor type-1 (PAI-1) in myoepithelial cells (MEs) may occur in early breast cancer lesions [45]. This strong expression of PAI-1 in MEs may result in the disruption of the interactions formed between urokinase plasminogen activator receptor (uPAR) expressed by MEs with vitronectin molecules within the basement membrane, thereby inducing their detachment of the basement membrane in high-grade breast ductal carcinoma *in situ* and apoptotic death [45]. In this matter, it will be important to establish whether certain prostatic and breast cancer progenitor cells and/or their early progeny localized in the basal compartment are also destroyed during the early stage of carcinogenesis or if they can survive against these insults. Moreover, the focal disruption of ME layers in the basal compartment during breast cancer progression has also been associated with a loss of estrogen receptor (ER) expression and an enhanced expression of several invasion-related genes in the overlying tumor cells [44]. This indicates that the normal basal epithelial cells might trigger a detachment from the basement membrane and apoptosis during cancer progression due to the changes in their local microenvironment that favor the tumor cell invasion.

**late-stage EMT program**

The progression to the locally invasive cancers implicates a degradation of the components of basement membrane including collagen, laminin, fibronectin and vitronectin by MMPs and uPA secreted by cancer cells and activated stromal cells (Figure 1) [16, 28, 46–48]. These molecular events may promote the invasion of migrating cancer cells into reactive stroma and their subsequent metastatic spread via the lymphatic vessels and systemic circulation at the near lymph nodes and distant tissues/ organs. The enhanced expression of different subsets of oncogenic genes in tumorigenic cancer progenitor cells and their further differentiated progeny in late-stage EMT program might also confer to them partial or more complete mesenchymal properties during the progression to locally invasive cancers (Figure 1) [26, 34]. In support with this, several recent lines of evidence have revealed that the invasive cancer types such as mammary, ovarian, prostatic, pancreatic, gastric, colorectal and squamous cell carcinomas may harbor an intratumoral heterogeneity with distinct proliferating and differentiating regions, including a preferential localization of migrating cancer progenitor cells in an EMT-like state at the invasive front (Figure 1) [9, 49]. Moreover, different subtypes of the precancerous lesions and invasive cancer forms have also been identified which are characterized by specific gene expression patterns and differentiation states [50–56]. For instance, at least five subtypes of breast cancer have been classified including invasive basal and basoluminal breast cancer subtypes which are characterized by distinct expression patterns of ER-α, progesterone receptor and erbB2 (also designated as HER-2 and neu) [54–56]. This may be associated with different genetic alterations and/or the activation of different tumorigenic cascades in cancer progenitor cell subpopulations during cancer initiation and/or progression. Furthermore, the changes within the local microenvironment of cancer progenitor cells during cancer progression may also influence their acquisition of a more malignant behavior. Hence, these molecular events may provide to cancer progenitor cells and their further differentiated progeny distinct oncogenic phenotypes within specific intratumoral regions and/or cancer subtypes (Figure 1). In support with this, it has been observed that the tumors derived from the induction of different initiating oncogenic events in transgenic mouse models of mammary neoplasia may show distinct morphological and architectural features, and the EMT process may occur only in certain neoplasm types. For instance, EMT occurred during
tumor progression in HRas- and Myc-induced mammary carcinomas as inferred by the expression of mesenchymal cell markers, while EMT was not detected in neoplasms arising in transgenic mice for erbB2 and Wnt-1 [57]. In addition, the targeted expression of stabilized β-catenin in the basal ME cell layer of mouse mammary epithelium has also been reported to result in an increase of proliferation and amplification of basal-type cell progenitors with abnormal differentiation and a lack of lineage markers that led to the development of invasive basal-type carcinomas [58]. Interestingly, it has also been noted that the ER-α-negative breast cancer cells, which did not express the metastasis-associated gene 3 (MTA3) that inhibits Snail transcriptional activity, may express a lower level of E-cadherin, and therefore possess a higher migratory capacity than the ER-α-positive breast cancer cells [59]. Similarly, it has been reported that a subgroup of aggressive glioblastoma multiform tumors (also designated as primary glioblastoma multiform), which represents a heterogeneous population of cancer cells, may arise from the malignant transformation of neural stem cells that acquire the mesenchymal properties like mesenchymal stem cells and give rise to further differentiated progeny [25, 26, 34]. In fact, these aggressive glioblastomas, which are frequently accompanied by the overexpression of EGFR, seem to develop rapidly without evidence of a transitory step of lower-grade tumor. In contrast, other glioblastoma multiform types (also termed secondary or progressive glioblastoma multiform), which are often characterized by the mutations in p53 suppressor gene, appear to derive from low-grade tumors that did not show the genetic changes associated with the EMT program [34]. Thus, on the basis of these observations, it appears that the stem cell-like properties of poorly- or moderately differentiated cancer progenitor cells did not represent their unique intrinsic feature that may influence their tumorigenic and migrating properties. In fact, the specific genetic alterations occurring in cancer progenitor cells and/or their progeny including acquisition of mesenchymal phenotypes during cancer initiation and progression may represent a more determinant factor of their aggressive and invasive properties (Figure 1). Hence, these differences noted between the expression pattern of mesenchymal genes in cancer progenitor cells and their progeny as well as the changes in their local microenvironments during the cancer progression may be responsible at least in part for the intratumoral heterogeneity and/or the development of different cancer subtypes with distinct levels of invasiveness. Regardless, we describe here the implication of diverse autocrine and paracrine loops stimulated by distinct growth factors, cytokines and integrins in tumor epithelial cells, activated fibroblasts and infiltrating inflammatory cells that may contribute to the sustained growth and survival of cancer cells during cancer progression (Figure 2). These survival factors may also reciprocally collaborate to induce a fuller EMT program and invasion process during the development of diverse aggressive cancers.

**functions of growth factors, cytokines and integrins in EMT process**

The interplay of diverse growth factors and cytokines secreted by the cancer cells and host cells concomitant with the changes in their local microenvironments may contribute to triggering the molecular events that are associated with the EMT program during the cancer progression [9, 28, 34, 35, 37]. Particularly, the enhanced expression of several growth factors like HGF, EGF, sonic hedgehog (SHH), Wnt ligands, stromal cell-derived factor-1 (SDF-1) and TGF-β and/or their receptors in cancer cells may result in an up-regulated expression of numerous gene products involved EMT process (Figure 1). Moreover, the secretion of diverse soluble factors such as HGF, EGF and TGF-β by host cells including activated fibroblasts, infiltrating immune cells and endothelial cells as well as the changes in the components of the basement membrane and ECM may also influence the cancer cell behavior including their growth, survival and migratory ability [9, 27, 28, 35, 37]. More specifically, the integrin-mediated adhesion of cancer cells to the surrounding ECM components in reactive stroma may trigger the intracellular signaling cascades that contribute to their acquisition of a migratory and invasive phenotype during EMT program [60–65]. Among the intracellular effectors activated in cancer cells through these growth factor and integrin signaling networks, there are the enhanced expression of several transcriptional repressors including Snail, Slug, smad interacting protein 1 (SIP1 as known as ZEB2) and/or activator of N-cadherin, Twist1 that may lead to a down-regulation of E-cadherin expression in several cancer cell types [9, 28, 30, 31, 33, 37, 41, 42, 66–68]. This may result in the disruption of intercellular adherens junctions formed by E-cadherin/β-catenin complexes and the invasion of cancer cells through reactive stroma. In addition to the disruption of adherens junctions, the activation of transcriptional repressors Snail, Slug and/or SIP1 may also lead to the simultaneous repression of the expression levels of distinct junctional proteins of tight-, gap- and/or desmosomal junctions in certain cancer cell types [27, 41, 42]. Hence, the sustained activation of multiple growth factors, cytokines and integrins in cancer cells during cancer progression may contribute to their uncontrolled growth, survival, scattering and acquisition of an increased motility and invasive ability by inducing the changes in intercellular adhesion junctions [9, 28, 34, 35, 37]. In regard with this, we discuss here the critical functions assumed by the growth factors, with a particular emphasis on the role of the EGFR–EGFR system, Wnt/β-catenin, SHH, TGF-β and integrin signaling pathways, in oncogenic transformation of cancer cells during the EMT program and invasion process.

**EGFR signaling.** The stimulation of EGFR signaling by its ligands EGF, TGF-α, heparin-binding EGF-like growth factor (HB-EGF) and amphiregulin, through autocrine and paracrine loops, may lead to the activation of Src, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, nuclear factor-kappaB, phospholipase Cγ (PLCγ) and β-catenin intracellular cascades and the up-regulation of MMP and uPA expression (Figures 1 and 2) [11, 12, 27, 34, 69–75]. This may contribute to the sustained growth, survival, migration and invasion of cancer progenitor cells during the development of diverse cancers such as brain, skin, lung, colon, pancreas, bladder, prostate, breast and ovarian carcinomas. More specifically, several studies indicated that the activation of EGFR signaling may result in the dissociation of cancer...
cells through the disruption of desmosomal and/or adherens junctions, and migration via the activation of Raf/MAPK, small GTPase Rac and/or PLCγ pathways (Figure 2) [27, 38]. In support of this, the blockade of EGFR signaling has been observed to promote the desmosomal assembly and intercellular adhesion in SCC68 squamous carcinoma cells [40]. Furthermore, EGF and Src may induce a tyrosine phosphorylation of the β-catenin found in the adherens junction in human carcinoma cells, thereby triggering cell dissociation [76]. In this matter, it has also been reported that EGF may induce an increase in the motility and the acquisition of a fibroblast-like morphology in ovarian surface epithelium cells, which are recognized as the precursors of ovarian carcinomas, through the activation of MAPK and integrin-linked kinase (ILK)/PI3K/Akt cascades and up-regulating activities of MMP-2 and -9 [77]. Interestingly, the characterization of human bladder carcinoma cell lines, CAL 29 and CAL 185, established from two patients with high-grade tumors, has also revealed that the tumorigenic CAL 29 cells expressed phenotypic features of both cell scattering and EMT after stimulation by EGF and TGF-α [78]. In contrast, nontumorigenic CAL 185 cells neither scattered nor expressed the mesenchymal marker vimentin in the presence of these growth factors [78]. Furthermore, it has been noted that CAL 29 cell scattering could be reversed by the removal of these growth factors, and that both scattering and EMT were inhibited after treatment with MEK, Src or PI3K inhibitor. This indicates that these signaling elements might be involved in triggering the EMT program in tumorigenic bladder cancer cells. The treatment of DU145 human prostatic cancer cells, which possess an intermediate phenotype, with EGF has also been observed to result in a disruption of cell–cell adhesion junctions by caveolin-1-mediated E-cadherin endocytosis concomitant with the release of a membrane E-cadherin-associated fraction of β-catenin into cytoplasm in the early phase after treatment (Figure 2) [79]. This was followed by the translocation of cytosolic β-catenin to the nucleus where it promoted lymphocyte enhancer factor (LEF)/T-cell factor (TCF) transcriptional activity. Moreover, the reforming cell–cell junctions could be prevented by the down-regulation of caveolin-1 in the late phase after EGF treatment. This event, in turn, led to the up-regulation of Snail-induced decreased E-cadherin and caused EMT in DU145 cells. In addition, the activation of the PLCγ signaling pathway by EGF may result in up-regulating uPA expression and, subsequently, to uPA secretion and its membrane uptake through uPAR. Thereby, this may also enhance the invasive properties of cancer cells [72].

The coexpression of the constitutively active EGFRvIII mutant with wild-type EGFR in diverse solid tumors, such as glioma, prostate, breast, lung, colorectal and ovarian carcinomas, may also contribute to the molecular events associated with the EMT process (Figure 2) [69, 80–82]. In support with this, it has been reported that the transfection of EGFRvIII into the OVCA 433 epithelial ovarian cancer cell line resulted in a cell dissociation and acquisition of a motile and fibroblastoid phenotype comparable to that observed in EGF-stimulated parental OVCA 433 cells [82]. More specifically, a disruption of adherens and desmosomal junctions was detected in EGFRvIII-expressing cells as indicated by the decreased levels of cellular plakoglobin and E-cadherin. In this matter, the integrin engagement by ECM components such as fibronectin may also lead to EGFR activation in the absence of ligand. Thereby, this may induce Ras/Erk and PI3K/Akt signaling that promotes the survival, migration and invasion of cancer cells (Figure 2) [83]. Hence, the EGFRvIII and integrins-induced ligand-independent activation of EGFR in matrix-adherent cells may enhance the cancer cell survival and invasion in the absence of ligand. Moreover, the EGF–EGFR axis may also cooperate with other growth factor signaling including Wnt/β-catenin, SHH, TGF-β and integrins for inducing a fuller EMT program. Particularly, the stimulation of EGF and TGF-β signaling may synergistically stimulate the EMT in the human malignant keratinocytes through the activation of MAPKs/AP-1 signaling which leads to an enhanced Smad2/3 transcriptional activity [84]. More specifically, these combined agents may induce the alterations at the level of intercellular connections by reducing the levels of the tight junction proteins claudin-1 and occludin and down-regulating E-cadherin at the adherens junctions [85]. Moreover, it has also been reported that EGF–EGFR and SHH/PITC/GLI signaling may cooperate to regulate the expression of specific target genes, proliferation and the invasion of normal and cancer epithelial cells [70, 86, 87]. In addition, the tight junction protein, claudin-1 may up-regulate MMP-1 and MMP-2, whose MMPs in turn may induce the cleavage of the laminin-5 γ2 chain [88]. This results in the binding of the laminin-5 γ2 chain to EGFR and/or integrin and the activation of intracellular cascades that promote cell growth and the motility of cancer cells. Hence, the up-regulation of EGFR signaling in cancer progenitor cells and/or their early progeny during the progression of numerous aggressive cancers may substantially contribute to all the steps in the EMT process, thereby promoting the development of more invasive and metastatic disease states.

**other growth factor, cytokine and integrin signaling.** The activation of the canonical Wnt/β-catenin signaling pathway in numerous cancers, including colorectal, GI, pancreatic, hepatocellular, mammary, prostatic, non-small-cell lung and skin basal cell carcinomas and melanoma, may result in the inhibition of intracellular β-catenin degradation and its nuclear accumulation (Figure 2) [6, 7, 11, 12, 27, 30, 33, 73, 89, 90]. Furthermore, the inactivating mutations in adenosomatous polyposis coli tumor suppressor gene or axin and/or the activating mutations in amino-terminal phosphorylation sites in cytoplasmic β-catenin molecules might also lead to the stabilization of β-catenin and translocation to the nucleus [89]. Hence, the nuclear β-catenin localization may result in up-regulating of β-catenin/LEF/TCF transactivation complex and concomitant expression of many targeted tumorigenic gene products [9, 12, 30, 89]. Among them, the enhanced expression of cyclin D1, c-Myc and survivin in cancer progenitor cells may provide to them high proliferative rate and resistance to apoptosis. Furthermore, other gene products, including the ECM proteins (osteopontin and tenasin-C), cell adhesion molecules (L1CAM and LAMC2) and Snail and Slug transcriptional repressors of E-cadherin expression, which are involved in the EMT process, may also promote the migration and invasion of tumor cells. Similarly, the activation of the
SHH/PTCH/GLI signaling cascade might also lead to the up-regulation of numerous tumorigenic target genes such as c-Myc, cyclin D1 and Snail (Figure 2). Thereby, this may promote the tumor growth, EMT, by inhibiting the E-cadherin expression and invasion in numerous aggressive and metastatic cancers including brain, GI, pancreas, breast, prostate, small-cell lung and basal cell carcinomas [7, 11, 12, 27, 70, 73, 90, 91]. Importantly, it has also been reported that the incidence of SHH-induced tumor formation (15%) in nestin-expressing neural progenitors in the cerebella of newborn mice was further enhanced by coexpression with IGF-II (39%), indicating that these oncogenic signaling pathways may cooperate during medulloblastoma ethiopathogenesis [92].

Although the fact that the overexpression of TGF-β in certain cancer epithelial cells and activated stromal cells may result in an inhibition of cell proliferation and/or apoptosis at early-stage carcinogenesis, this cytokine can rather promote the tumor invasion and metastasis at later stages [28, 29, 33, 49, 93–95]. This may be associated with the changes in the expression and/or activity of TGF-β signaling effectors or cross talks with other activated antiapoptotic and tumorigenic cascades during cancer development. In this matter, TGF-β secreted by cancer cells and activated fibroblasts can cooperate with several other survival growth factor signaling including RTKs, Wnt/β-catenin, Notch and oncogenic Ras for inducing a fuller EMT in malignant epithelial cells [29, 30, 32, 35, 93, 96, 97]. For instance, the activation of TGF-β signaling combined with the enhanced stimulation of Ras induced through EGF and platelet-derived growth factor (PDGF) signaling or the expression of oncogenic mutant Ras may lead to the hyperactivation of MAPKs and/or a PI3K/Akt cascade in many cancer cells including pancreatic, breast, prostate, skin and liver cancer cells in late-stage tumorigenesis (Figure 2) [30, 35, 93, 97]. In this matter, a recent study has also revealed that depletion of interleukin-related protein (ILEI) in human cancer cells could prevent TGF-β-induced EMT [98]. In addition, human Cripto-1, which is a member of the EGF-Cripto-FRL1-Cryptic family that may act as a membrane-associated coreceptor for Nodal, a TGF-β superfamily member, is also overexpressed in numerous cancers including cervix, colon, stomach, pancreas, lung, breast, ovary and testis [99, 100]. The enhanced expression of Cripto-1 also appears to be associated with the malignant transformation of epithelial cells and EMT and can promote their proliferation, migration and invasion during tumorigenesis and metastasis.

On the other hand, the activation of growth factor and ECM component/integrin signaling pathways in cancer cells may also lead to changes in the expression of focal adhesion molecules such as ILK, focal adhesion kinase (FAK) and the scaffolding proteins, like Crk-associated substrate and paxillin, and MMPs during the EMT process that contribute to the cell dissociation and migration (Figure 2) [27, 30, 60–62, 64, 83, 101–104]. More specifically, the activation of β1- or β3-integrin on cancer cells by binding to stromal ECM components such as type IV collagen, laminin and fibronectin may lead to the activation of numerous cascades, including Ras, Rac, PI3K, ILK, β-catenin and/or PLCγ signaling elements, in cancer cells that contribute to their proliferation and survival as well as the disruption of cell–cell interactions and migration during EMT (Figure 2) [60–64, 101]. For instance, it has been reported that the binding of type IV collagen to β1-integrin on the surface of breast cancer cells results in the activation of FAK/paxillin/MAPK signaling that leads to the destabilization of intercellular adhesion structures and cancer cell migration [65]. Additionally, MMPs and disintegrin and metalloproteinases can also activate numerous tumorigenic signaling cascades in cancer epithelial cells through the release of soluble ligands such as EGF, TGF-α, HB-EGF, IGF, TGF-β and c-KIT from their cell surface-associated latent precursors and thereby promote tumor invasion (Figure 2) [46–48, 103]. More specifically, MMP-7 (also known as matrilysin), which is overexpressed in a variety of invasive cancers including GI, pancreas, colorectal, liver, lung, skin, breast and prostate carcinomas, may induce the proteolytic shedding of E-cadherin [103]. This results in the release of the ectodomain of E-cadherin in the pericellular space, the disruption of adherens junctions, thereby promoting cancer cell invasion. Hence, the acquisition of a migratory fibroblastoid phenotype by cancer progenitor cells and their progeny during the EMT program induced through the complex interactions of distinct growth factor signaling pathways appears to represent an important event that may contribute to development of locally invasive and metastatic cancers.

metastatic cancer

As in primary neoplasm, the tumor initiation at host distant tissues/organs may also result from the division of the tumorigenic and migrating cancer progenitor cells or their early progeny from primary neoplasm that give rise to further differentiated cell subpopulations (Figure 1) [9, 10, 16, 22, 28, 105]. At the present time, the molecular events that govern the preferential migration and adhesion of cancer progenitor cells at specific metastatic sites as well as their adaption of a quiescent or activated state are not yet precisely known. The tumor growth and differentiation status of metastatic neoplasms appear to depend on several factors intrinsic and extrinsic to tumorigenic cancer progenitor cells, including the local dynamic microenvironment prevalent in their novel homing site. In this matter, several lines of evidence have also indicated that the metastatic spread of invasive cancer progenitor cells could be influenced by their intrinsic properties which have been acquired during primary neoplasm development [106–108]. In support with this, a specific gene expression pattern in primary breast tumor cells from patients has notably been associated with the incidence of lymphatic or bone marrow micrometastases [52, 54, 108]. Moreover, the analyses of 117 primary breast tumors during 5-year follow-up of the patients’ outcomes have also revealed that a specific expression pattern of gene products could be associated with the incidence of distant metastasis [106]. More recently, it has also been reported that a 70-gene signature might provide additional prognostic information on distant metastasis-free survival probability for patients with early breast cancer [52, 54]. Hence, it appears that each individual tumorigenic cancer progenitor cell and/or their early progeny in primary neoplasm must acquire an oncogenic phenotype that confer to them the capacity to reach the circulation and survive within lymphatic and blood vessels, and migrate to distant tissues/organs where they may establish their...
novel homing. In this matter, the acquisition of a migratory behavior by the tumorigenic cancer progenitor cells may represent an important factor for their invasion and metastases at distant sites. In addition, the tumor-initiating capacity of cancer progenitor cells and/or their early progeny at a particular metastatic site may be influenced by the transforming effects of other adjacent cells within multicellular angiogenic clusters forming the micrometastatic unities. As in the primary tumor, host stromal cells including fibroblasts, infiltrating inflammatory cells and endothelial cells can all collaborate reciprocally with cancer progenitor cells to each step of the micrometastases and tumor formation at metastatic sites [9, 16, 28, 104, 105, 109]. Furthermore, certain cancer cells may also undergo a mesenchymal–epithelial transition at the specific metastatic sites indicating that the new local microenvironment prevalent at certain metastatic tissues/organisms may reverse their migratory phenotype acquired during the EMT program in primary invasive neoplasm [9, 29, 37].

This indicates that the oncogenic phenotype specific to each metastatic tumor-initiating cell as well as their local microenvironment, as in the primary tumor, may represent important factors that determine the growth rate and differentiation pattern of metastatic tumors. Regardless, certain lines of evidence have also revealed that the degree of differentiation of metastatic cancer cells might directly influence their tumorigenic potential and the architecture of tumors established in vivo. For instance, the metastatic and androgen-independent PC3 and DU145 prostate cancer cell lines, which possess an intermediate phenotype [CK5+/18+, CD44+ and low or undetectable androgen receptor (AR)], are more tumorigenic than the metastatic AR-positive LNCaP cell line characterized by a luminal phenotype (CK5−, CD44− and CK8/18−) [11, 20, 110]. Moreover, the PC3 cells with an intermediate phenotype form poorly differentiated tumors in vivo show the architectural features like the patient’s metastatic original tumor. In addition, it has been shown that certain established human cancer epithelial cell lines may represent a heterogeneous population of cancer cells, and the presence of a little subpopulation of tumorigenic progenitor cells expressing stem cell-like markers may be responsible for their capacity to form the tumor and metastasize animal models in vivo with a high occurrence. For instance, it has been observed that CD44-positive DU145, -LAPC-4 or -LAPC-9 cancer progenitor cell subpopulations purified from the cultured cells or xenograft tumors, which show higher expression levels of β-catenin and hedgehog signaling element, Smothened (SMO) than the corresponding CD44-negative cell subpopulation, was more proliferative, clonogenic, tumorigenic and metastatic in vitro and in vivo [20, 21]. Similarly, the analyses by flow cytometry-based side population Hoechst technique of cultured human cancer cells revealed that ~30% of the cancer cell lines examined possessed a detectable side population. More specifically, the side population cells purified from U373 glioma, MCF7 breast and LAPC-4 prostate cancer cell lines, which show the stem cell-like intrinsic properties and expressed some stemness genes including Notch-1 and β-catenin, were also more tumorigenic than the corresponding nonside population cells in vivo [21]. Further studies should allow us to establish whether the presence of a subpopulation of cancer progenitor cells with the stem cell-like properties within available transformed cell line models correlate with their tumorigenic and/or metastatic potential in vivo.

Together these observations indicate that the heterogeneity noticed between the differentiation patterns and architectures of different cancer subtypes as well as in distinct regions within an even tumor type may be associated with the presence of distinct tumor-initiating cancer progenitor cells with the specific structural and functional properties (Figure 1). Furthermore, the local microenvironment of cancer progenitor cells prevalent at the primary and metastatic sites also appear to constitute a determinant factor that may influence the development of the primary tumor and progression from micrometastasis into well-established metastatic cancers.

**novel therapeutic strategy against aggressive cancers**

The acquisition of an invasive phenotype during the EMT program by cancer cells in skin, liver, lung, prostate, breast, ovarian, pancreas, GI and colorectal cancers is often associated with the development of resistance to current therapeutic treatments and a poor outcome of patients [9, 15, 28, 66, 67, 111–115]. Therefore, several novel therapeutic strategies have been investigated for counteracting the progression from premalignant lesions into locally invasive and metastatic forms, thereby preventing disease relapse [11, 12, 22, 108, 116, 117]. Among these new approaches, the induction of the differentiation of cancer progenitor cells by using the agents such as retinoic acid and its synthetic analogues or histone deacetylase inhibitor which induce the differentiation, growth arrest and/or apoptotic death of cancer cells may represent a promising chemopreventive strategy for numerous cancers including AML, colorectal, bladder and hepatocellular cancers [2, 15, 118–120]. Furthermore, the molecular targeting of the oncogenic signaling elements involved in the malignant transformation of adult stem cells and/or their early progeny into tumorigenic and migrating cancer progenitor cells represents promising strategies to prevent, treat and even reverse the disease. More specifically, the blockade of growth factor, cytokine and integrin pathways that lead to the activation of signaling elements such as β-catenin and transcription factors, Slug, Snail and Twist1 that assume the critical roles during the EMT program and invasion process may constitute potent strategies to counteract the progression from localized cancers into disseminated diseases [6, 7, 11, 22, 71, 73, 90, 121–126]. In this matter, we describe novel therapeutic strategies based on targeting of oncogenic signaling elements frequently activated in tumorigenic and migrating cancer progenitor cells and their local microenvironment during the EMT program in localized cancers.

**molecular targeting of oncogenic signaling elements in cancer progenitor cells**

The blockade of oncogenic cascades including EGFR, hedgehog and/or Wnt/β-catenin, which are recognized to play a crucial role in the malignant transformation of cancer progenitor cells and/or their early progeny during the cancer progression
from preinvasive lesions into localized invasive and metastatic cancers, is particularly promising (Figure 3) [11, 12, 16, 34]. In this regard, numerous studies have indicated that the blockade of these tumorigenic signaling pathways may result in the inhibition of the growth and apoptotic death of invasive and metastatic cancer cells in vitro and in vivo. Particularly, it has been observed that the blockade of the EGFR signaling pathway by using the anti-EGFR antibody or EGFR tyrosine kinase inhibitor such as gefitinib or erlotinib resulted in a cell cycle arrest in the G1 phase, an inhibition of invasion and/or induced apoptosis in numerous metastatic cancer cell types in vitro and in vivo [11, 12, 70, 72, 74, 120, 127–132]. Moreover, it has been reported that the use of a tyrosine kinase inhibitor such as erlotinib, which is able to inhibit wild-type EGFR and mutant EGFRvIII or combined monoclonal antibodies 528 and 806 directed against EGFR and EGFRvIII, respectively, may represent the more effective treatments for cancer overexpressing these two EGFR forms (Figure 3) [81, 133–135]. Importantly, it has also been reported that gefitinib or EGFR/erbB2 inhibitor, GW2974, was able to prevent and counteract the development of gall-bladder carcinoma in the BK5.erbB2 transgenic mouse model in vivo [136]. Similarly, the use of a tyrosine kinase activity inhibitor such as PKI-166, TAK165, GW572017 (lapatinib) or CI-1033, which targets several erbB family members including EGFR and erbB2 also constitutes an alternative strategy against certain aggressive cancer subtypes expressing these different receptors (Figure 3) [11, 12, 70, 73, 120, 126, 130, 137, 138]. In addition, the inhibition of the hedgehog cascade, by using the SMO signaling element inhibitor, cyclopamine alkaloid or the anti-SHH antibody, has also been observed to result in an inhibition of the growth and invasion of metastatic cancer cells in vitro and in vivo, while the normal epithelial cells were insensitive to the cytotoxic effects of these agents (Figure 3) [7, 11, 12, 70, 73, 123, 125]. Furthermore, the inhibition of Wnt/β-catenin signaling by using a selective Wnt antibody, Wnt protein inhibitors or repressors disrupting nuclear TCF/β-catenin complexes may also be beneficial for improving the treatments against certain invasive cancers, and more particularly colorectal cancer in which this oncogenic cascade is involved (Figure 3) [12, 139]. It has also been noticed that the simultaneous inhibition of these distinct tumorigenic cascades is generally more effective than the blockade of unique signaling [11, 12, 70, 73, 129].

In addition, the inhibition of the oncogenic cascades activated during the EMT program at late stages of carcinogenesis may also constitute a promising approach to counteracting the metastatic spread of invasive cancer cells. As a matter of fact, the inhibition of autocrine PDGF/PDGFR loop-induced EMT by overexpressing a dominant-negative PDGFR construct or imatinib mesilate (STI571), which is a potent inhibitor of α and β-PDGFRs and other tyrosine kinases, caused apoptosis in human mammary carcinoma cell lines and decreased the

Figure 3. Novel therapeutic strategies against aggressive and invasive cancers by targeting distinct growth factor signaling cascades in cancer progenitor cells and their progeny. The possible antiproliferative, anti-invasive and/or apoptotic effects induced by the tyrosine kinase activity inhibitors including epidermal growth factor receptor (EGFR) (gefitinib), EGFR/EGFRvIII (erlotinib) and EGFR/erbB2/erbB3 (CI-1033) as well as by a selective inhibitor of Smoothened (SMO) hedgehog signaling element (cyclopamine) and monoclonal antibody directed against sonic hedgehog (SHH), Wnt, tumor growth factor (TGF)-Rβ or β1-integrin are indicated.
incidence of metastasis in nude mice in vivo [97]. Similarly, the
down-regulation of the TGF-β cascade by using a selective TGF-
βR kinase inhibitor, such as SD-093 and SD-208, anti-TGF-β or
-TGF-βR antibody or antisense compounds, also inhibited
tumor growth and/or metastasis of diverse cancer cell lines in
athymic nude mice in vivo (Figure 3) [93, 95, 140]. Moreover,
the inhibition of the β1-integrin signaling in the breast cancer
cells by using selective anti-β1-integrin antibody AIIB2 also
resulted in an inhibition of the tumor growth in vivo and
apoptotic death of the cells in vitro, while nonmalignant cells
were resistant to this treatment type (Figure 3) [141]. Of
particular interest, the targeting of Cripto-1 oncofetal gene
product or tumor ECM component tenasin-C, which are
strongly expressed during cancer progression and involved in
the EMT process by using monoclonal anti-Cripto or anti-
tenasin C antibody, also represent alternative approaches for
the treatment of numerous invasive and metastatic cancers
[100, 142].

molecular targeting of the tumor
microenvironment of cancer progenitor cells

Although the tumorigenic and migrating cancer progenitor
cells may assume a pivotal role in cancer progression, further
differentiated tumor cells, normal basal cells and stromal cells
in angiogenic clusters may also influence their malignant
transformation and the tumor development. Therefore, the
design at the basis of new effective curative treatments for
aggressive cancers should also consider the cancer progenitor
cells as an integral part of the multicellular unit which may be
influenced by the changes in their local microenvironment. In
this matter, recent lines of evidence have indicated that several
epithelial cancer types, such as GI, pancreatic, colorectal, lung,
prostatic, breast and ovarian cancers, could derive from
precancerous lesions occurring during sustained tissue injuries,
such as chronic proliferative inflammatory atrophy [7, 10–14,
28, 143, 144]. Therefore, the suppression of inflammatory
responses and/or angiogenesis by using the nonsteroidal anti-
inflammatory drugs, selective inhibitor of cyclooxygenase-1 or 2
(COX-1 or -2), NF-kB and/or vascular endothelial growth factor
(VEGF)–vascular endothelial growth factor receptor (VEGFR)
may also constitute other strategies for decreasing the incidence
of these cancer types [126, 144–152]. As a matter of fact, it has
been reported that the targeting of both VEGFR1 and VEGFR2-
positive circulating endothelial cells by selective neutralizing
antibodies blocked tumor angiogenesis and induced tumor
necrosis [153]. Importantly, it has also been observed that the
treatment of squamous cell carcinoma cell A-5RT3 xenografts
established in nude mice in vivo with a monoclonal anti-
VEGFR2 antibody resulted in an inhibition of tumor growth
[143]. Moreover, this antitumoral effect also led to the reversion
of a highly invasive carcinoma into a premalignant dysplastic
lesion [143]. This was also accompanied by a reduction of both
the endothelial cell proliferation and vascularization and
stromal expression of MMPs within the tumor as well as a
reconstitution of the regular basement membrane. This
indicates that the blockade of the VEGF–VEGFR transduction
system represents a promising strategy for simultaneously
inhibiting angiogenesis and MMPs proteolytic activity, thereby
preventing and/or counteracting tumor growth. Moreover,
since certain lines of evidence have revealed that the endothelial
cells that are involved in tumor vascularization might express
different set of genes as compared with angiogenic endothelial
cells from nonmalignant tissues, the targeting of tumor-specific
angiogenesis markers is also of particular interest. In support
with this, it has notably been reported that the targeting of
markers specifically overexpressed on tumor endothelium,
including vimentin, CD59, HMGB1 and IGBP7, inhibited
angiogenesis in vitro and in vivo [154]. Recent investigations
have, however, revealed that the antiangiogenic strategies may
cause hypoxia, which, in turn, may lead to a positive selection of
tumor cells with a high oncogenic phenotype that are more
resistant to ionizing radiation and chemotherapy. Therefore, the
combination of antiangiogenic strategy with bioreductive
agents, such as N-oxides, quinines and nitroaromatics
that target tumor hypoxia, ionizing radiation and/or
chemotherapeutic drugs targeting tumor cells, may constitute
the most effective therapeutic approaches for counteracting
cancer development [149, 151, 155]. On the other hand, since
the interaction between the SDF-1 released by stromal
endothelial cells and fibroblasts with its receptor CXCR4
expressed on numerous cancer cell types assumes a crucial role
for inducing their survival, invasion/EMT and recruitment and
adhesion at lymph nodes and distinct metastatic sites including
bone marrow, the blockade of SDF-1-CXCR4 axis may also
constitute a possible strategy to prevent the vascular invasion
and metastasis formation [12, 68, 102, 114, 156–159].

combination therapy

Recent works carried out to identify new targeting strategies for
improving the current cancer therapies against the aggressive
cancers have revealed the substantial benefits of developing
combination drug therapies as compared with monotherapy
[11, 12, 69, 70, 127–130]. The combination therapy by targeting
different oncogenic signaling elements can reduce the secondary
effects associated with the use of high doses of cytotoxic drugs.
It can also improve the poor survival rate and disease relapse
observed with the current antihormonal, radiotherapy and
chemotherapeutic treatments, which remain yet ineffective
against most aggressive and metastatic cancer forms [11, 160,
161]. In this matter, since the overexpression of the EGFR
and/or its ligands occur during the development of numerous
aggressive cancer types including brain, skin, prostate, breast,
ovary and small-cell lung cancers and is often associated with
the recurrence of disease, the blockade of EGFR is of particular
therapeutic interest [11, 12, 34]. In support with this, the
preclinical trials with a low dose of oral active gefitinib have
notably indicated a potential benefit of using this type of agent
which generally shows a good bioavailability and little side-
effects, in combination with other chemotherapeutic drugs
[131, 162]. Moreover, gefitinib has been observed to enhance
the antitumoral effects induced by the antiangiogenic agents
such as bicalutamide as well as diverse chemotherapeutic agents
including platinum compounds, cisplatin and carboplatinum
and paclitaxel on vulvar, lung and prostate cancer cells in vitro
and in vivo [163–165]. Another study has also indicated that the
combined anti-EGFR antibody (cetuximab) and anti-VEGFR2


antibody, DC101 induced a synergistic antitumor effect on the BxPC-3 and GEO pancreatic cell xenografts established in animal models in vivo [161]. Furthermore, the results of our recent works have revealed that the combined use of lower doses of a selective EGFR inhibitor such as gefitinib or PD153035 with cyclopamine, ER inhibitor (tamoxifen), protein kinase A inhibitor (Rp-cAMP) or an activator of ceramide production such as etoposide and docetaxel were more effective for inducing the growth arrest and apoptosis of metastatic and androgen-sensitive LNCaP-C33 and -independent LNCaP-C81, DU145 and PC3 cells as compared with the individual drugs [70, 75, 127, 128, 166]. Similarly, the results from a recent investigation have also revealed that EGFR activation may be responsible for the development of the resistance of MCF-7 breast cancer cells to tamoxifen treatment by inducing the EMT program [167]. It was also found that the use of gefitinib could inhibit the invasion and migration of tamoxifen-resistant cells [167].

In addition, the activation of the IGF/IGF-IR autocrine loop in certain cancer cell types, which leads to the stimulation of the PI3K/Akt survival pathway, appears to be responsible for the resistance to chemotherapeutic drug-induced cytotoxicity, indicating that the blockade of this cascade may also be of therapeutic interest [168]. For instance, since at least 70% of small-cell lung cancers overexpressing the KIT receptor have active IGF-R1 signaling which can protect the cancer cells against the growth inhibitory effect mediated through the stem-cell factor/KIT transduction system, it appears that the simultaneous inhibition of these two cascades could be a more effective treatment of this disease. As a matter of fact, the data from a recent study have revealed that the combined use of selective inhibitors of IGF-IR (NVP-ADW742) and tyrosine kinase activity, imatinib (STI571), was more potent than individual drugs at inhibiting the growth of small-cell lung cancer cells in vitro [113]. Moreover, it has been reported that the NVP-ADW742 and imatinib might enhance the sensitivity of small-cell lung cancer cells to cytotoxic effects induced by etoposide and carboplatin [115]. Additionally, since the up-regulated expression of Twist has been associated with the resistance of nasopharyngeal, bladder, ovarian and prostate cancer cells to taxol, its molecular targeting may also represent a new strategy to overcome the acquired cellular resistance [169]. As a matter of fact, it has been observed that the down-regulation of Twist in metastatic and androgen-independent DU145 and PC3 cells improved their sensitivity to taxol-induced apoptotic death, and suppressed their migration and invasion capacities by up-regulating E-cadherin expression [170].

conclusions and perspectives

Altogether, these recent investigations revealed that the tumorigenic cancer progenitor cells may play a crucial role for tumor growth and their acquisition of a migratory phenotype during the EMT program may provide to them a highly invasive and metastatic behavior which is important for their metastasis at distant sites. Hence, the resistance of these tumorigenic and migrating cancer progenitor cells with stem cell-like properties to current clinical cancer therapies may contribute to the poor outcome of patients and disease relapse. Therefore, the determination of oncogenic cascades activated in the cancer progenitor cells versus their further differentiated progeny at the primary and metastatic cancer sites is of particular therapeutic interest. This should allow us to identify new potential biomarkers and therapeutic targets for the development of more effective diagnostic and prognostic methods and the treatment of aggressive and recurrent cancers in the clinics. More particularly, the establishment of novel combination therapies by targeting both cancer progenitor cells and their further differentiated progeny as well as the altered components within their local microenvironment should eliminate the total cancer cell population, and thereby prevent the recurrence of the disease.

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