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Pushing tumor cells towards a malignant phenotype: Stimuli from the microenvironment, intercellular communications and alternative roads

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Mini Review

The tumor microenvironment produces different types of stimuli capable of endowing tumor cells with an aggressive behavior that is characterized by increased motility, invasiveness and propensity to metastasize, gain of a tumor-initiating phenotype, and drug resistance. The following classes of stimuli have been reported to promote such a malignant phenotype: (*i*) solid- or fluid-induced stress; (*ii*) altered composition of the extracellular matrix; (*iii*) hypoxia and low pH; (*iv*) innate and adaptive immune responses; (*v*) antitumor drugs. The simultaneous presence of more than one of these stimuli, as likely occurs *in vivo*, may lead to synergistic interactions in the induction of malignant traits. In many cases, the gain of a malignant phenotype is not the result of a direct effect of the stimuli on tumor cells but, rather, a stimulus-promoted cross-talk between tumor cells and other cell types within the tumor microenvironment. This cross-talk is mainly mediated by two classes of molecules: paracrine factors and adhesion receptors. Stimuli that promote a malignant phenotype can promote additional outcomes in tumor cells, including autophagy and cell death. We summarize here the available evidence about the variables that induce tumor cells to take one or the other of these roads in response to the same stimuli. At the end of this review, we address some unanswered questions in this domain and indicate future directions of research.

Key words: malignant, stimuli, epithelial-mesenchymal transition, cell death, autophagy

Abbreviations: AMPK: 5′ adenosine monophosphate-activated protein kinase; BCC: breast cancer cell; CAF: cancer-associated fibroblast; CCL: chemokine (C–C motif) ligand; CCR: chemokine (C–C motif) receptor; CXCL: chemokine (C–X–C motif) ligand; CXCR: chemokine (C–X–C motif) receptor; ECM: extracellular matrix; EMT: epithelial-to-mesenchymal transition; HIF: hypoxia-inducible factor; HMGB1: high-mobility group protein B1; IFP: interstitial fluid pressure; IL: interleukin; MDSC: myeloid-derived suppressor cell; miR: microRNA; MMP: matrix metalloproteinase; MSC: mesenchymal stem cell; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NSCLC: non-small cell lung cancer; TGF: transforming growth factor; TLR: toll-like receptor; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor

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The Malignant Phenotype

Primary human tumors are characterized by unlimited replicative potential and genomic instability.[1,2] Over a large number of cell divisions this leads to genetic diversity, thereby promoting the propensity of tumor cells to respond to microenvironmental factors with an increased degree of phenotypic plasticity.[2] This explains why, during primary growth, some tumor cells can acquire traits that endow them with a malignant phenotype, that leads to increased tumor cell motility, invasiveness and propensity to metastasize, gain of a tumor-initiating phenotype and drug resistance. In many cases, tumor cells undergo coordinated expression of these traits.[3] In particular, epithelial-to-mesenchymal transition (EMT) leads to the acquisition of a phenotype that encompasses all these traits. EMT is characterized by down-regulation of epithelial markers and up-regulation of mesenchymal markers.[4] These changes lead to loss of cell polarity and adhesion, and gain of motile characteristics. As a result, EMT promotes the detachment of cells from the primary tumor, facilitating their migration and metastatic dissemination.[4] Experimental evidence also suggests a strong link between EMT, drug resistance,[4,5] and acquisition of a tumor-initiating phenotype.[6–9] It is to note, however, that EMT is not an "all-or-nothing" event, but manifests over a spectrum of phenotypic and functional changes,[10–14] and is also a transient condition that can revert once the affected tumor cells home to sites of

metastatic seeding.[15] Thus, malignant traits may become detectable in a non-concerted fashion.

Stimuli that Promote a Malignant Phenotype

Tumor cells can become more aggressive as a result of genetic changes[16,17] or in response to extrinsic factors and deriving from the tumor microenvironment. By reviewing the literature, we have identified the following stimuli (Table 1): (*i*) solid- fluid-induced stress; (*ii*) altered composition of the extracellular matrix (ECM); (*iii*) hypoxia and low pH; (*iv*) innate and adaptive immune responses; (*v*) antitumor drugs. All these stimuli originate from the tumor microenvironment, although one of them (antitumor drugs) is administered exogenously. Their common denominator is that they represent various forms of cellular stressors. In the following, we discuss available evidence for their role as promoters of malignant traits.

Solid- and fluid-induced stress

Most solid tumors are characterized by high interstitial fluid pressure (IFP) owing to increased vascular permeability and lymphatic impairment,[63] by elevated solid pressure due to uncontrolled proliferation of tumor cells in a confined space,[63] and by interstitial flow owing to abnormal pressure differentials between tumor and surrounding normal tissue.[64] These abnormalities, which generally coexist, have negative consequences on the uptake and penetration of antitumor drugs[65–67] and, in addition, promote the acquisition of malignant traits by tumor cells. Thus, elevated solid stress inhibits apoptosis,[19] induces mammary carcinoma cells to acquire an invasive phenotype,[20] promotes EMT[21] and alters the composition of the ECM.[22] The level of IFP correlated with metastatic dissemination and treatment resistance in patients with human cervical carcinoma.[18] Abnormal interstitial flow promoted glioma cell invasion.[64] Most recently, fluidic stream has been reported to induce EMT in ovarian cancer nodules. This was driven in part by a posttranslational up-regulation of epidermal growth factor receptor expression and activation.[24]

Altered composition of the ECM

Solid tumors possess an abnormal ECM characterized by a network of connective tissue molecules, like collagen fibers, denser than in normal tissues.[23] This renders tumors stiffer than normal tissues, thereby causing alterations of the mechanical properties of the tumor stroma that are conducive to a malignant phenotype.[68,69] Indeed, increased stiffness of the ECM was found to alter cell–cell and cell–matrix interactions, and to induce hyaluronan synthesis and expression of genes involved in invasion and metastasis.[22,25–27] A stiffer ECM may cause elevation of integrin-mediated cytoskeletal tension in malignant breast tissue which, in turn, can drive focal adhesions, disrupt adherens junctions, perturb tissue polarity[28] and promote invasion and metastatic dissemination.[29] Experiments performed with an artificial ECM allowing microfabrication of channels of defined wall stiffness and geometry showed that human glioma cells migrate faster in ECM with elevated stiffness and narrow channels. This behavior was attributed to increased polarization of cell-stroma traction forces.[30]

Altered composition of tumor stroma can induce a malignant phenotype also by activating intracellular signaling pathways. Thus, collagen-induced activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)[31] and autocrine transforming growth factor (TGF)-β signaling in non-small cell lung cancer (NSCLC) cell lines[32] promoted EMT. Elevated production of hyaluronan, another major component of the tumor stroma, induced EMT and drug resistance in NSCLC[33] and human breast cancer cells[34] through binding to its receptor, CD44.[34,70]

From a clinical perspective, high breast tissue density was associated with a greater than fourfold increased risk of developing breast carcinoma, making it one of the greatest independent risk factors for breast cancer.[71]

Hypoxia and low pH

Proliferation of tumor cells forces blood vessels apart and reduces delivery of oxygen to vessel-distant tumor cells. This leads to activation of anaerobic glycolysis and accumulation of metabolic products, such as lactic and carbonic acid, which lower the extracellular pH.[72,73] Thus, hypoxia and low pH are often concomitant. Hypoxia, however, can be present at physiologic pH,[74] and aerobic glycolysis in tumors (the Warburg effect) can lead to accumulation of metabolites that lower pH in the absence of hypoxia.[75] Both hypoxia and low pH can contribute to the acquisition of a malignant phenotype, and the simultaneous presence of both has synergistic effects in several tumor types.[76–79]

In the presence of an acidic extracellular pH, protons were shown to diffuse from the proximal microenvironment of breast and colon cancer xenografts into adjacent normal tissues where they caused tissue remodeling that favored local invasion.[43] An acidic pH promoted invasiveness, metastatic dissemination and drug resistance in several melanoma xenografts.[44] In addition, expression of proton pumps that enhance extracellular acidosis was increased in chemoresistant human epidermoid and prostate cancer cells and upon administration of chemotherapeutic drugs.[80]

Regarding hypoxia, many of its effects are due to reactive oxygen species[35]-dependent up-regulation of the transcription factors hypoxia-inducible factor (HIF)-1 and 2α, which lead to the expression of many genes, including genes encoding paracrine factors.[81–83] Thus, hypoxia and HIFs promote tumor angiogenesis, accelerate tumor growth, motility and invasion, formation of premetastatic niches and metastatic dissemination,[36,37] induce resistance to radiation and chemotherapy,[38,39] genetic instability[40–42] and EMT.[35] Hypoxia can induce EMT and the accompanying malignant traits also in hematological malignancies, like multiple myeloma.[84]

Table 1. Stimuli that promote a malignant phenotype in tumor cells

Class	Subclass	Consequences (examples)	Reference
Mechanical stress	High IFP	• Promotion of metastatic dissemination and treatment resistance	18
	High solid stress	• Inhibition of apoptosis • Induction of mammary carcinoma cells to acquire an invasive phenotype • Promotion of EMT • Up-regulation of adhesion molecules and induction of hyaluronan synthesis	19–24
	Abnormal interstitial flow/fluidic stream	• Promotion of glioma cell invasion • Promotion of EMT in ovarian cancer nodules	
Altered ECM	Increased stiffness of the stroma	• Alteration of cell–cell and cell–matrix interactions, induction of hyaluronan synthesis and expression of genes involved in invasion and metastasis • Elevation of integrin-mediated cytoskeletal tension in malignant breast tissue → disruption of adherens junctions, perturbation of tissue polarity • Promotion of tumor cell invasion and metastatic dissemination	22,25–29
	Narrow channels in artificial ECM mimicking tumor stroma	• Faster migration of tumor cells	30
	Elevated density of individual stroma components	• Collagen-induced activation of NF-κB and TGF-β signaling promoting EMT in NSCLC cell lines • Elevated hyaluronan production inducing EMT and drug resistance	31–34
Hypoxia and low pH	Hypoxia	• Promotion of tumor angiogenesis, accelerated tumor growth, motility and invasion, formation of premetastatic niches and metastatic dissemination, induction of resistance to radiation and chemotherapy, genetic instability, and EMT	35–42
	Acidic extracellular pH	• Induction of tissue remodeling favoring local invasion • Induction of invasiveness, metastatic dissemination and drug resistance in melanoma xenografts • Recruitment of myeloid-derived suppressor cells (MDSC) promoting escape from immune surveillance, inducing neo-angiogenesis and stromal remodeling	43–45
Antitumor drugs	Cytotoxic drugs	• Induction of drug resistance in surviving tumor cells • Induction of paracrine factors promoting a malignant phenotype through recruitment of monocytes	46–49
	Ionizing radiation	• Induction of tumor-initiating cell surface and embryonic stem cell (TLR) markers, capacity to self-renew, and generation of differentiated progeny, mesenchymal phenotype	50–56
	Biological drugs	• Angiogenesis inhibitors accelerating tumor growth and metastasis formation, increasing frequency of tumor-initiating cells through induction of hypoxia, increasing lymphatic and distant metastasis formation • Anti-VEGF antibody bevacizumab inducing malignant traits through the induction of paracrine factors recruiting myeloid cells and promoting EMT in an autocrine manner	
Innate and adaptive immune responses	Inflammatory stimuli engaging Toll-like receptors	• *Listeria monocytogenes* and *Helicobacter pylori*promoted growth of gastric carcinoma through TLR2 and TLR4 signaling • Engagement of TLR4 promoting tumor growth and inducing chemoresistance • Single-stranded RNAs engaging TLR7/8, inhibiting apoptosis, increasing tumor cell survival and chemoresistance	57–59
	Sterile inflammation	• Promotion of tumor growth and metastatic dissemination by high-mobility group protein B1 (HMGB1)	60
	Antigen-specific immune responses	• Induction of EMT, stem cell properties, and drug resistance • Promotion of metastasis formation by regulatory T cells	61,62

Antitumor drugs

Chemotherapeutic drugs are a mainstay in tumor therapy. It may appear paradoxical, therefore, that they represent also efficient tools to promote a malignant phenotype. As to the mechanism whereby chemotherapeutics induce malignant traits, it has been shown that head and neck cancer cells[46] and cells of the prostate cancer microenvironment[85] surviving treatment with cytotoxic drugs played an important role through the secretion of drug-induced paracrine factors. In lung carcinoma cells, the drug resistance and antitumor effects induced by chemotherapy depended on different intracellular signaling pathways,[86] with drug resistance being mediated by paracrine factors released from tumor-associated macrophages and tumor cells. Release of paracrine factors mediating drug resistance can be the result of a complex interaction between different players. As an example, two chemotherapeutics (gemcitabine and 5-fluorouracil) caused lysosomal release of cathepsin B from tumor cells, which induced activation of the inflammasome, release of interleukin (IL)-1β and IL-1β-induced release of IL-17 from CD4-positive T cells. IL-17 then acted as final effector in the induction of chemotherapy resistance.[47] Drug-induced paracrine factors can promote a malignant phenotype also through the recruitment of cells, like monocytes, as it has been described for mouse mammary and human breast cancer cells.[48,49]

Also non-chemotherapeutic drugs, including ionizing radiation and biologics, can promote gain of malignant traits. Thus, NSCLC cells surviving radiation, expressed markers of tumor-initiating cells and embryonic stem cells. These cells were able to self-renew, generate differentiated progeny and had a mesenchymal phenotype.[50] Angiogenesis inhibitors accelerated tumor growth and metastasis formation in murine models,[51] increased the frequency of tumor-initiating cells in human breast cancer xenografts through induction of intratumoral hypoxia[52,53] and increased lymphatic and distant metastasis formation in mouse models of pancreatic cancer and glioblastoma.[54] The anti-vascular endothelial growth factor (VEGF) antibody bevacizumab induced malignant traits in a mouse model of pancreatic cancer through the induction of paracrine factors that recruited myeloid cells[55] and promoted EMT.[56] In a large number of cancer cell lines, resistance of B-Raf-mutant melanoma to Raf inhibitors was shown to be due to stromal cell secretion of hepatocyte growth factor (HGF) and activation of its specific receptor MET.[87,88]

Innate and adaptive immune responses

Both innate and adaptive immune responses can promote a malignant phenotype. Regarding innate immune responses, Toll-like receptors (TLR) are known to play a key role as sensors for the recognition of invading pathogens. Infectious agents like *Listeria monocytogenes* and *Helicobacter pylori* have been shown to promote tumor growth of gastric carcinoma through TLR2 and TLR4 signaling, respectively.[57] Engagement of TLR4 promoted growth and induced chemoresistance in cells from ovarian cancer patients and ovarian cancer cell lines.[58] Single-stranded RNAs engaged TLR7/8, leading to up-regulation of the anti-apoptotic protein B cell lymphoma gene-2, increased tumor cell survival and chemoresistance in malignant lung tissues and human lung cancer cell lines.[59] Tumor-secreted and exosome-transported microRNA (miR)-21 and miR-29a bound to human TLR8 on immune cells, thereby triggering an inflammatory response that ultimately promoted tumor growth and metastatic dissemination.[89]

Also sterile inflammation[90] that ensues as a result of acute or chronic cell damage due to exogenous (*e.g.*, trauma) or endogenous insults (*e.g.*, chronic inflammatory diseases, ischemia, *etc.*) has been shown to induce malignant traits.[91] Induction of malignant traits by sterile inflammatory responses is largely mediated by proteins called "alarmins," which are either released upon necrotic cell death or actively secreted by myeloid cells.[90]

Regarding adaptive immune responses, CD4-positive, immunosuppressive regulatory T cells and chronically stimulated CD8-positive effector T cells can produce cytokines that induce tumor cells to undergo EMT, acquire tumor-initiating cell properties and enhance resistance to drugs and radiation.[61] Another study reported that regulatory T cells promoted metastasis formation in rat mammary carcinomas through release of the receptor activator of NF-κB ligand.[62]

Interactions Between Different Players Involved in the Promotion of a Malignant Phenotype

Different stimuli acting in concert

In a tumor, several of the non-genetic factors that act as stimuli in promoting a malignant phenotype are present at the same time. Thus, as already discussed, hypoxia is often accompanied by low pH, and increased solid stress may cause collapse of tumor blood vessels and, consequently, hypoxia. One may ask which are the consequences of different stimuli acting in concert. Available data suggest the possibility of synergistic interactions. Thus, concomitance of hypoxia and low pH synergistically promote gain of malignant traits.[76] Synergistic interactions were also documented between altered ECM composition and tumor hypoxia that maximized tumor progression and cooperated to drive metastatic dissemination.[92]

Stimuli stimulating each other

Although each of the five classes of stimuli that we have listed can promote a malignant phenotype, individual stimuli can also induce one or more stimuli belonging to other classes. As already mentioned, solid stress can generate hypoxia as well as modify the composition of the tumor microenvironment by increasing tumor cell synthesis of hyaluronan.[22] Figure 1*a* shows a schematic view of the

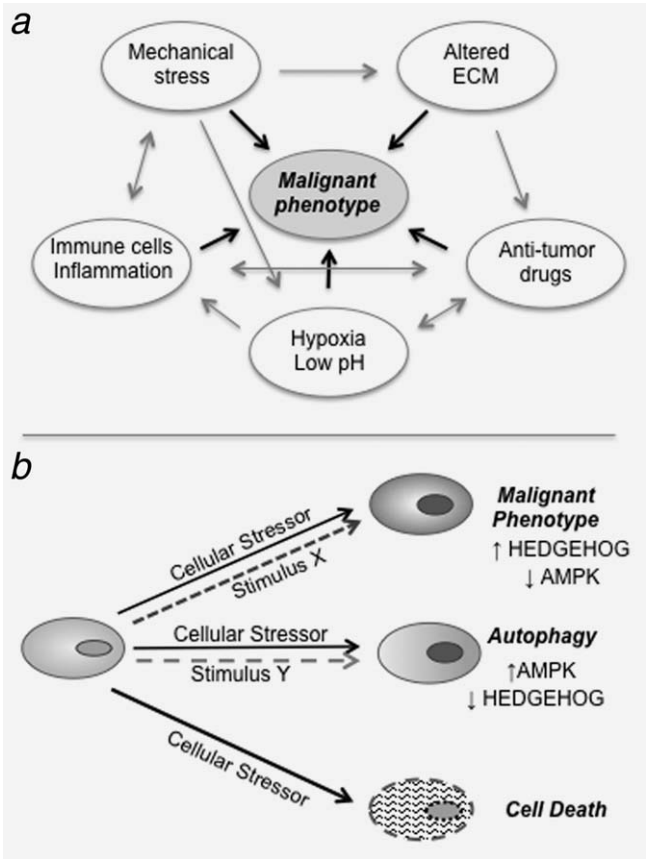

Figure 1. Interactions promoting a malignant phenotype and alternative roads. (*a*) The cartoon depicts the interactions between different stimuli that have been reported to promote a malignant phenotype, and between these stimuli and tumor cells. Bold arrows indicate the direction of the effects on tumor cells. Thin arrows indicate the interactions between stimuli. Examples of these interactions are given in the text. (*b*) Model to explain how tumor cells can acquire a malignant phenotype, undergo autophagy or cell death in response to the same stimulus/cellular stressor. Tumor cells may acquire a malignant phenotype in response to one of the cellular stressors listed in the text, and an undefined stimulus X. The interaction between these two factors leads to activation of Hedgehog signaling and/or inhibition of AMPK signaling. Alternatively, tumor cells can undergo autophagy in response to the same cellular stressor and an undefined stimulus Y (*e.g.*, nutrient starvation?). These modifications are accompanied by activation of AMPK signaling and/or inhibition of Hedgehog signaling. Eventually, tumor cells undergo cell death when the cellular stressor exceeds a given threshold of intensity and/or duration.

interactions between the different classes of stimuli in promoting a malignant phenotype.

Cross-talk between different cell types in the promotion of a malignant phenotype

Promotion of a malignant phenotype, while depending in many cases on the presence of either one or more of the stimuli that we have described, is generally the result of a cross-talk between tumor cells and other cell types of the tumor microenvironment (Table 2): *that is*, mesenchymal cell types, such as vascular endothelial cells,[93] cells of bone marrow origin (including tumor-associated macrophages[94] and myeloid-derived suppressor cells [MDSC][49], mesenchymal stem cells (MSC),[95] myofibroblastic cancer-associated fibroblasts (CAF)[96,97] and others. The interplay between these cells and tumor cells can be very complex, as has been shown in breast cancer models.[98]

The go-between: paracrine factors and adhesion receptors

The cross-talk between different cell types within the tumor microenvironment that leads to the acquisition of malignant traits is largely mediated by paracrine factors and adhesion receptors.

Paracrine factors. A large number of paracrine factors, of diverse chemical nature, including peptides (*e.g.*, thymosin β4),[102] chemokines,[58] cytokines,[103] growth factors,[104] enzymes like matrix metalloproteinases (MMP),[8] lysyl oxidase,[105] furin,[106] cathepsins,[107] nitric oxide,[108] lactate and ketone bodies[100] and fatty acids,[99] have been reported to mediate the cross-talk between different cell types of the tumor microenvironment (Tables 2 and 3). IL-6,[109,110] tumor necrosis factor (TNF)-α,[111] TGF-β,[112,113] chemokine (C–C motif) ligand (CCL) 9,[114] chemokine (C–X–C motif) ligand (CXCL) 1/2,[49] CXCL10[98] and CXCL12[115] are among the most frequently reported cytokines and chemokines, respectively.

An emerging class of paracrine factors involved in the promotion of malignant traits is alarmins. Thus, HMGB1, and members of the calgranulin family like S100A8/A9, has been shown to promote accelerated tumor growth, metastatic dissemination, establishment of a premetastatic niche and induction of chemoresistance in several different models of solid tumors like hepatocellular carcinoma, gastric cancer and mesothelioma.[49,60,91,116–119]

Some paracrine factors can synergize in the induction of malignant traits, as it has been shown for TGF-β1 and TNF-α.[120] Moreover, paracrine factors can also induce the synthesis of other molecules that contribute to a malignant phenotype, like TGF-β and IL-1β in the induction of hyaluronan in NSCLC cell lines.[33]

Overall, the network of paracrine factors acting as mediators of malignant traits appears highly redundant, and experimental evidence obtained with cell lines from different solid tumor types supports this view.[121]

Integrins and other adhesion receptors. Altered composition of the ECM is accompanied by up-regulation of adhesion molecules[18] and adhesion receptors, like integrins.[122] Integrins link ECM components with intracellular cytoskeletal components and elicit intracellular signaling when clustered or ligand-occupied. There is now considerable evidence that the altered composition and expression of adhesion molecules and integrins may contribute to the induction of a malignant phenotype. Thus, it has been reported that solid stress up-regulates the expression of β_1 integrin and

Table 2. Cross-talk between different tumor cells types: participating cell types and paracrine mediators (selected examples)

Stimulus	Step 1	Step 2	Step 3	Effect	Reference
Hypoxia	Recruitment of MSCs	MSCs augmenting HIF activity and inducing expression of CXCR3, CCR5, and placental growth factor in breast cancer cells (BCC)	BCCs-induced expression of CXCL10, CCL5, and VEGF receptor 1 in MSCs	Enhancement of invasion and metastasis formation of BCCs to the lungs and lymph nodes. Further recruitment of MSCs	98
Hypoxia	Cell-free conditioned medium containing the chemokine CCL2 derived from hypoxic mammary tumor cells	Injection of mice with the conditioned medium	Increased bone marrow-derived cell infiltration (predominantly $CD11b^{+}$/$Ly6C^{med}$/$Ly6G^{+}$ and $CD3^{-}$/$NK1.1^{+}$ cells) into the lung in the absence of a primary tumor	Creation of premetastatic niche and increased metastatic burden	36
Chemotherapeutic drugs (gemcitabine, 5-fluorouracil)	Activation of the inflammasome in MDSCs	IL-1β secretion due to lysosomal permeabilization and release of cathepsin B, its binding to the inflammasome and caspase-1 activation	IL-1β inducing secretion of IL-17 by CD4-positive T cells	Induction of resistance to chemotherapy and promotion of tumor growth	47
Chemotherapeutic drug (doxorubicin)	Doxorubicin killing cancer cells and inducing TNF-α production by endothelial and other stromal cells	Cancer cells overexpressing CXCL1/2 attract $CD11b^{+}Gr1^{+}$ myeloid cells producing chemokines, including S100A8/9	TNF-α enhancing CXCL1/2 expression in cancer cells, thereby amplifying the CXCL1/2-S100A8/9 loop	Induction of chemoresistance and priming for survival in metastatic sites	49
Chemotherapeutic drugs (mitoxantrone, docetaxel)	Activation of NF-κB in cells of the prostate cancer microenvironment	Secretion of a spectrum of proteins including WNT16B	Paracrine activation of the canonical Wnt program in tumor cells	Attenuation of the effects of cytotoxic chemotherapy *in vivo*, promotion of tumor cell survival and disease progression	85
Chemotherapeutic drugs (platinum analogs)	Activation of MSCs	Induction of polyunsaturated fatty acids, 12-oxo-5,8,10-heptadecatrienoic acid and hexadeca-4,7,10,13-tetraenoic acid (16:4(n-3))	Polyunsaturated fatty acids acting on tumor cells	Induction of resistance to a broad spectrum of chemotherapeutic agents	99
Antitumor drugs (tamoxifen, doxorubicin)	Coculture of estrogen receptor-positive BCCs with CAFs	Induction of L-Lactate and ketone bodies in CAFs	Up-regulation of TP53-induced glycolysis and apoptosis regulator in BCCs	Induction of drug resistance in BCCs	100
Inflammatory stimulus (lipopolysaccharide)	Stimulation of mouse macrophage cell line RAW 264.7	Induction of various proinflammatory cytokines, including TNF-α and IL-1β	Stimulation of lung cancer cells in combination with TGF-β	Proinflammatory cytokine-mediated enhancement of TGF-β-induced EMT	101

Table 3. Paracrine factors as mediators of a malignant phenotype

Classes of mediators	Individual mediators (examples)	References
Peptides	Thymosin β4	102
Chemokines	CCL9	114
	CXCL1/2	49
	CXCL12	115
Cytokines	TGF-β	113
	IL-6	93
	TNF-α	111
Alarmins	HMGB1	60
	Calgranulins	49
Enzymes	MMPs	8
	Lysyl oxidase	105
	Furin	106
	Cathepsins	107
Nitric oxide		108
Lactate and ketone bodies		100
Fatty acids		99

other genes involved in glioblastoma and breast cancer cell invasion.[27] Increased stiffness of the tumor stroma was accompanied by enhanced β_1 integrin expression and metastatic outgrowth in a model of breast cancer.[123] Adhesion of small cell lung cancer cells to ECM enhanced tumorigenicity and conferred resistance to chemotherapeutic agents as a result of β_1 integrin-stimulated tyrosine kinase activation suppressing chemotherapy-induced apoptosis.[124] Stiffening of the ECM led to integrin clustering, increased invasion of a premalignant mammary epithelium,[125] and enhanced growth of experimental breast cancer.[28] Fibrosis associated with deposition of type I collagen induced breast cancer cells to form proliferative metastatic lesions mediated by β_1 integrin.[126]

Not surprisingly, interactions between paracrine factors and adhesion receptors have been described in the induction of a malignant phenotype. Thus, interaction between β_3 integrin and TGF-β receptor II facilitated TGF-β-mediated oncogenic signaling, EMT and metastasis in models of human breast cancer.[127,128] Others had previously shown that TGF-β-induced EMT is dependent on functional β_1 integrin.[129]

When considering the role of integrins in the promotion of a malignant phenotype, a contradictory aspect becomes apparent: overexpression of integrins enhances adhesion of tumor cells to the ECM, yet it promotes gain of malignant traits, such as metastatic dissemination, which require enhanced motility. Increased adhesion, however, may be considered as a first step, which is followed by loss of tumor cell polarity and adhesion, allowing tumor cells to gain enhanced motility along a stiffer ECM with narrower channels,[31] thereby leading to faster migration, invasion and metastatic dissemination.

Another class of adhesion receptors that influence acquisition of a malignant phenotype is cadherins. EMT is typically accompanied by down-regulation of E-cadherin, an epithelial marker, and up-regulation of N-cadherin, a mesenchymal marker. Interestingly, modulation of the expression of these cadherins is not a mere reflection of the transition, but contributes actively to EMT. Thus, ectopic E-cadherin expression inhibited invasion of melanoma cells by down-regulating invasion-related adhesion receptors, melanoma cell adhesion molecule and integrin β_3, and induced apoptosis in melanoma cells *in vitro*.[130] Up-regulation of N-cadherin, conversely, enhanced expression of fibroblast growth factor receptor, resulting in EMT and stem/progenitor like properties.[131]

Become Aggressive, Rest or Die: A Difficult Choice

It is noteworthy that the same stimuli that promote a malignant phenotype can also cause tumor cell death or autophagy. Autophagy is a lysosomal degradation pathway that allows cells to cope with stressors by destroying damaged proteins and organelles.[132] It can be considered as a defensive response promoting tumor cell survival.[133] Examples of stimuli that can promote both cell death and malignant traits are solid stress, low pH, hypoxia and chemotherapeutics.[86,134–137] Examples of stimuli that can induce autophagy include hypoxia, genotoxic stress and low pH.[132,138–140] Thus, similar stimuli can induce tumor cells to become aggressive (promotion of a malignant phenotype), enter a resting state (autophagy) or undergo cell death. It is of obvious interest to elucidate the variables that dictate tumor cells to take one road or another in response to the same stimulus. In the following, we will briefly summarize the available evidence.

An important variable that may dictate the choice between different modes of responses is the intensity of the stimulus. In several instances it has been observed that cell death can ensue when the stimulus is strong; when the stimulus is moderate, the cell can acquire a malignant phenotype. Thus, high solid stress suppressed cell proliferation and induced apoptotic or necrotic death of breast and lung cancer cells,[134,135] while lower solid stress led to gain of malignant traits in breast cancer cells.[21]

A second variable is the duration of the stimulus. One of the effects of transient exposure to low pH in the tumor microenvironment was the induction of anergy in melanoma-associated T cells, but a longer exposure to the same low pH led to apoptotic cell death.[136]

A third variable depends on the way tumor cells and/or cells of the tumor microenvironment respond to the same stimulus. As already discussed, the same stimulus may induce death of tumor cells and secretion of paracrine factors promoting malignant traits in cells of the tumor microenvironment.[48,49]

Eventually, another variable is related to the ability of a given cell type to deliver a deadly hit or promote the acquisition of malignant traits. Thus, antigen-specific lymphocytes

may differentiate either into cytotoxic cells that kill antigen-positive tumor cells, or into immunosuppressive cells that promote gain of a malignant phenotype.[61]

Regarding the choice between induction of a malignant phenotype and autophagy, recent evidence suggests that these two modes of responses correlate with the activation of different intracellular signaling pathways and gene expression programs. For example, activation of Hedgehog signaling has been shown to promote the acquisition of malignant traits,[141,142] while its inhibition favored autophagy.[143] Activation of 5′ adenosine monophosphate-activated protein kinase (AMPK), conversely, reduced tumor cell invasion and release of paracrine factors,[74] suppressed EMT induction in non-neoplastic renal tubular epithelial cells[144] and promoted autophagy in response to genotoxic stress[133] and nutrient starvation.[132] Inhibition of AMPK, conversely, allowed tumor cells to maintain energy production under nutrient deprivation, preserve cytoskeletal dynamics and activate the cell motility effector focal adhesion kinase, thereby inducing enhanced tumor cell invasion and metastatic dissemination.[145] Thus, it appears that tumor cells are skewed toward a malignant phenotype upon inhibition of AMPK signaling, whereas active signaling promotes autophagy.[132,146]

On the basis of this knowledge, we propose the following model to explain how tumor cells can be induced to take one of the three roads (Fig. 1*b*). According to this model, the combination of a cellular stressor and a second stimulus (indicated in the figure as X or Y) promotes a malignant phenotype or autophagy, respectively. In a variant of this model (not shown in the figure), one response (*e.g.*, gain of a malignant phenotype) may be the result of the cellular stressor(s) acting alone, whereas the other response (*e.g.*, autophagy) is the result of a cellular stressor acting in concert with a second stimulus. According to both variants of the model, promotion of a malignant phenotype can be the result of activation of Hedgehog signaling and/or inhibition of AMPK signaling, whereas the reverse may apply for the promotion of autophagy. In both cases, tumor cell death would ensue when the intensity or duration of the stimuli exceeds a threshold level.

Of note, Vismodegib, an inhibitor of Hedgehog signaling, has recently gained approval for the treatment of advanced basal cell carcinoma,[147] although results from clinical trials in other more common types of solid tumors have been disappointing.[148] According to the model that we have proposed, one possibility to explain these negative results is that inhibition of Hedgehog signaling may promote the induction of autophagy, thereby favoring the survival of tumor cells and their resuming growth after a period of quiescence.

Points to Consider for the Future

In this review, we have proposed a classification of stimuli from the tumor microenvironment that promote a malignant phenotype. Although our knowledge in this field has expanded considerably over the last decade, many issues deserve further investigation. In the following, we indicate those that we feel are most relevant to foster our understanding in this field.

As already mentioned, tumor cells can acquire a malignant phenotype as a result of tumor-associated genomic instability. This can lead to the overexpression of molecules (*e.g.*, chemokines, cytokines and their receptors, regulators of apoptosis) that can directly promote a malignant phenotype, bypassing the requirement for external stimuli.[17,149] Little information, however, is available regarding the relevance of one or the other, or both mechanisms (*i.e.*, genetic *vs.* microenvironmental) in promoting a malignant phenotype in individual tumors and different tumor types. Remarkably, the tumor microenvironment itself constitutes a significant source of genetic instability. Thus, hypoxia and other adverse conditions of the tumor microenvironment are associated with the induction of mutagenesis, numerous types of DNA damage, fragile sites and gene amplification as well as dysregulation of DNA repair pathways.[150,151] This suggests the possibility of synergistic interactions and positive feed-back loops between the two mechanisms in inducing malignant traits. Clearly, more information on this aspect is desirable.

A second issue is as to whether the stimuli that we have listed and the malignant traits that are promoted by these stimuli are relevant for all tumor types. Although the stimuli and related phenotypic changes discussed in this review seem to involve the most commonly investigated tumor types of epithelial origin, there are clear differences between different tumor types regarding the impact of individual phenotypic changes to the overall malignant behavior and therapeutic response. For example, pancreatic adenocarcinoma has a prominent ECM that is very rich in hyaluronan and highly resistant to drug penetration[152]; breast cancer has a significant fraction of the stroma consisting of fibroblasts[153]; tissue elasticity and hydraulic conductivity vary depending on tumor.[154] Eventually, malignant traits may differ not only depending on the tumor type, but also within individual tumor types depending on their location[155] and tumor size.[156]

Finally, regarding the stimuli that can promote a malignant phenotype, we have included in our classification those that have been reported in the literature for tumors. However, changes similar to those that characterize a malignant phenotype can take place also in normal cells. Thus, EMT can be driven in normal cells not only by the same stimuli that we have described for tumor cells (*e.g.*, hypoxia),[157,158] but also by stimuli that have not yet been reported for tumor cells, like angiotensin II, aldosterone, albumin,[144] nickel[159] and inducers of ER stress.[160,161] It would be interesting to test whether some of these other stimuli may also contribute to the induction of a malignant phenotype in tumor cells.

Conclusions

Research on stimuli from the tumor microenvironment that induce malignant traits is raising increasing interest. We have proposed a tentative classification of these stimuli, and

identified paracrine factors and integrins as two main classes of mediators that are involved in the promotion of a malignant phenotype. Acquisition of a malignant phenotype is one of the possibilities a tumor cell has to respond to stressors, being autophagy or cell death the other two. We have also proposed some variables that promote induction of cell death rather than malignant phenotype, and identified two signaling pathways (Hedgehog and AMPK) that preferentially promote a malignant phenotype and autophagy, respectively. Expanding knowledge in this field appears especially desirable because of the significant fallout that this would entail in the diagnosis and therapy of cancer. In fact, research aimed at the identification of drugs that inhibit either the stimuli promoting a malignant phenotype or their effects appears a promising opportunity for new antitumor therapies. Some approaches are already being pursued[162–164] and it is likely that this stream of research will be expanded considerably in forthcoming years.

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