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The tumor macroenvironment and systemic regulation of breast cancer progression

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ABSTRACT Breast cancer is the most common malignancy among women worldwide and is the most common cause of death for women between 35 and 50 years of age. Women with breast cancer are at risk of developing metastases for their entire lifetime and, despite local and systemic therapies, approximately 30% of breast cancer patients will relapse (Jemal *et al.*, 2010). Nearly all breast cancer related deaths are due to metastatic disease, even though metastasis is considered to be an inefficient process. In some cases, tumor cells disseminate from primary sites at an early stage, but remain indolent for protracted periods of time before becoming overt, life-threatening tumors. Little is known about the mechanisms that cause these indolent tumors to grow into malignant disease. Because of this gap in our understanding, we are unable to predict which breast cancer patients are likely to experience disease relapse or develop metastases years after treatment of their primary tumor. A better understanding of the mechanisms and signals involved in the exit of tumor cells from dormancy would not only allow for more accurate selection of patients that would benefit from systemic therapy, but could also lead to the development of more targeted therapies to inhibit the signals that promote disease progression. In this review, we address the systemic, or "macroenvironmental", contribution to tumor initiation and progression and what is known about how a pro-tumorigenic systemic environment is established.

KEY WORDS: *metastasis, dormancy, systemic instigation, microenvironment*

Disseminated tumor cells

Most deaths from solid tumors are caused by haematogenous spread of cancer cells into distant organs and their subsequent growth to overt metastases. In general, minimal residual disease is defined as the presence of tumor cells, after surgical removal of the primary tumor, that are not detectable by the current routine diagnostic procedures used for tumor staging in cancer patients, but only become apparent after a period of time. A variety of terms are used in the literature to describe metastatic cells in blood and bone marrow. Tumor cells in visceral organs or bone marrow are most often referred to as disseminated tumor cells (DTCs), and those in the peripherial blood are termed circulating tumor cells (CTCs) (Pantel *et al.*, 2008).

The traditional view was that metastaic spread is a late process in malignant progression, in which a single cell of origin within the original clone acquires genetic variability, allowing sequential evolution of a more aggressive subline of cells (Nowell, 1976). This traditional view, often referred to as the clonal evolution of metastasis model, has been supported by studies demonstrating that copy number profiles in breast cancer primary tumours are highly similar to the metastatic tumors analyzed from these patients, suggesting that metastatic cells emerge from an advanced clonal expansion, and not from an earlier intermediate or a subpopulation different from the bulk of the primary tumor (Navin *et al.*, 2011). The opposing view is that dissemination of primary cancer cells to distant sites is often an early event, particularly during breast cancer progression. In fact, tumor cells have been detected in the circulation and bone marrow of patients with the early tumor type ductal carcinoma *in situ* (Husemann *et al.*, 2008). Molecular genetic analysis revealed that disseminated tumor cells often display genetic alterations that are distinct from the primary tumor (Ding *et al.*, 2010, Husemann

Abbreviations used in this paper: BMC, bone marrow cell; BMDC, bone marrow derived cell; CTC, circulating tumor cell; DTC, disseminated tumor cell; GRN, granulin; OPN, osteopontin.

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et al., 2008, Klein, 2009). These findings suggest that metastatic cells either diverge from the original tumor as new mutations are acquired during their progression or that metastases arise from a small minority of cells within the primary tumor.

Although the early steps of invasion and metastatic spread might be efficient, experimental models and clinical observations indicate that successful metastatic outgrowth, termed colonization, is quite an inefficient process (Luzzi *et al.*, 1998). Studies suggest that 0.01% of CTCs can ultimately produce a single bone metastasis, and at least 10,000 CTCs are required for the development of a metastatic colony (Panteleakou *et al.*, 2009). In addition, CTCs have been found in disease-free breast cancer patients up to 20 years after successful treatment (Klein, 2009). Thus, the presence of CTCs is a mandatory, but not sufficient, step in the generation of distant metastasis. In these situations, it is not clear why some CTCs/DTCs are able to give rise to metastases and others are not.

Dormancy and indolent cancers

The length of time required for the development of metastatic disease suggests a period of dormancy before DTCs are able to grow into clinically relevant metastases. The Australian pathologist Rupert Willis originally coined the term "dormant tumor cells" having conducted autopsy studies to analyze the metastatic spread of human cancers. In 1934 he wrote (p. 114):

> *When long-delayed metastatic tumors appear in a patient in whom there is no local recurrence of the extirpated primary growth, it is clear that the secondary growths must have arisen from tumor-emboli disseminated from the primary growth before its removal. The neoplastic cells must have lain dormant in the tissues in which they were arrested, and their resumption of growth must be attributed to some alteration in the qualities of these tissues or to some release of growth-restraints exercised by them on tumor cells. The nature of these factors is wholly unknown, and it is for future research to explain the remarkably sudden change of behaviour exhibited by the tumors in the cases under discussion* (Willis, 1934).

This concept was updated twenty years later by Geoffrey Hadfield, who introduced the idea of a "*temporary mitotic arrest*" to describe the prolonged latency periods of otherwise fully malignant tumor cells (Hadfield, 1954). Current experimental models have led to the concepts of cellular dormancy and tumor dormancy. The former argues that dormancy can be accounted for by the fact that disseminated tumor cells are in a state of mitotic arrest (Aguirre Ghiso *et al.*, 1999, Aguirre-Ghiso, 2007, Aguirre-Ghiso *et al.*, 2003, Aguirre-Ghiso *et al.*, 2001). Tumor dormancy, on the other hand, describes the situation during which the rate of cell death counterbalances the rate of cell proliferation within a tumor mass (Almog *et al.*, 2006, Naumov *et al.*, 2006). These two general forms of latency are not mutually exclusive; theoretically, both types of dormancy could coexist in the entire DTC population of a particular cancer patient.

While the general connotation of "dormancy" implies reference to metastatic disease, primary tumors can also undergo a period of latency until their clinical diagnosis. For example, women with primary breast cancer often return to the clinic with additional ipsilateral or contralateral primary breast tumors that were not detected at the time of initial diagnosis (Panet-Raymond *et al.*, 2011a, Panet-Raymond *et al.*, 2011b). Moreover, autopsy studies of young men and women with no medical history of cancer uncovered a surprisingly high number of cancers that had been clinically unapparent within the general population (Black and Welch, 1993, Folkman and Kalluri, 2004). An autopsy study of women between the ages of 40-49 revealed that nearly 40% of these women had some type of incipient breast tumor, yet only 2% of all women in this age category are ever diagnosed with breast cancer (Nielsen *et al.*, 1987, Sakr *et al.*, 1995). Similarly, prostate carcinoma *in situ* was surreptitiously discovered in as many as 31% of men aged 60-70 years who died of trauma but is clinically diagnosed in only approximately 8% of men in this age group (Sanchez-Chapado *et al.*, 2003). Most strikingly, microscopic carcinoma (often less than 1 mm in diameter) was found in the thyroid of more than 38% of individuals 41 to 60 years of age who died of trauma, but is diagnosed in only 0.5% of individuals in this age group (Harach *et al.*, 1985). In these cases, *cancer dormancy* is clearly a protracted stage in tumor progression in which primary tumors remain occult and patients are asymptomatic for a prolonged period of time. It is not known whether primary tumor dormancy is mechanistically different from metastatic dormancy, as the former is defined by controlled or unsuccessful growth within the natural habitat of the transformed cell, while DTCs must adapt to a new microenvironment.

The fact that DTCs must adapt to the environment of the metastatic site may partially explain why metastastic colonization is an inefficient process. A lack of proper growth signals and cell--to-cell signalling attachements was shown to lead to dormancy of DTCs in vivo, suggesting that a foreign microenvironment, with improper cell contacts and signaling, can lead to tumor cell dormancy (Aguirre Ghiso *et al.*, 1999). Conversely, tumor cells that have the ability to establish proper heterotypic interactions in their new environment have been shown in experimental models to form successful metastases (Shibue and Weinberg, 2009). In addition, nascent metastastic outgrowths must obtain a blood supply in order to grow to a significant size. Although cells in a primary tumor may be competent to induce angiogenesis, DTCs that have left the primary site before acquisition of this trait would ostensibly need to acquire intrinsic angiogenic ability or receive help from other sources, such as their microenvironment (Aguirre--Ghiso, 2007, Almog, 2010). Furthermore, surveillance by cells of the immune system can block the expansion and proliferation of DTCs, and thus this immunosurveillance must be evaded for the development of an overt metastasis (Aguirre-Ghiso, 2007, Eyles *et al.*, 2010). Though the challenges facing disseminated tumor cells at a metastatic site have been described, the mechanisms by which tumor cells overcome these challenges are just starting to be elucidated.

Systemic instigation

A growing body of evidence supports the notion that the tumors that co-exist in a patient who has multiple tumor burden can interact systemically to modulate overall cancer progression (Kim *et al.*, 2009, McAllister and Weinberg, 2010, Mullen *et al.*, 1985, O'Reilly *et al.*, 1997, O'Reilly *et al.*, 1994). Indeed, indolent human breast cancer cells ("responders") that are disseminated to various anatomical locations within host mice can be stimulated to form malignant tumors by systemic factors, namely cytokines and bone marrow-derived cells (McAllister *et al.*, 2008). These systemic signals are provided by aggressively growing human

breast tumors ("instigators") located at anatomical sites distant from the disseminated responding tumors. Instigating tumors exert their influence on the responding tumors from a distant anatomical location without metastasizing, or "self seeding", to the sites where indolent tumors reside (Kim *et al.*, 2009). Therefore, the process by which one tumor stimulates the distant growth of another is termed "systemic instigation."

The process of systemic instigation is evocative of earlier reports demonstrating that multiple tumors within a mouse host could affect one another from a distance. For instance, multiple tumor burden was shown to enhance the growth of otherwise latent cancers (Mullen *et al.*, 1985); in these studies, the presence of an immune-suppressor tumor growing in one anatomical site enabled the progression of otherwise-weakly tumorigenic foci at distant sites. In some experimental mouse isograft studies, anti-angiogenic factors secreted by a subcutaneous tumor inhibit the outgrowth of lung metastases by indirectly increasing apoptosis in tumor cells (Gohongi *et al.*, 1999, Holmgren *et al.*, 1995). On the other hand, it has been described that some tumors release pro-angiogenic factors, which induce mobilization of hematopoietic and endothelial precursor cells from the bone marrow into the circulation to support angiogenesis (Heissig *et al.*, 2002, Moore *et al.*, 2001, Orimo *et al.*, 2005, Rafii, 2000).

Collectively, clinical and experimental findings support the notion that primary tumors prior to their resection or metastatic colonies that have gained a growth advantage after surgical removal of the primary tumor can establish a pro-tumorigenic systemic environment to promote the progression of disseminated micrometastases that are poised to respond to these signals. It is becoming increasingly clear that many aspects of tumor progression can only be explained by a detailed understanding of both paracrine and systemic signaling cascades.

Mediators of systemic instigation

Implicit in the concept of systemic instigation is the notion that tumor activation of the host systemic environment is separable from response of tumors to the host systemic environment, or "macroenvironment". Establishment of the pro-tumorigenic systemic environment is mediated in part by the cytokine osteopontin (OPN), which is elevated in the plasma of patients with metastatic cancers and predictive of poor outcome (Mor *et al.*, 2005, Rudland *et al.*, 2002, Tuck *et al.*, 2007). OPN is necessary but not sufficient for the instigation process, indicating that other tumor-derived factors are required for the process (McAllister *et al.*, 2008). It is clear that indolent tumor cells are the ultimate beneficiaries of systemic instigation, but the systemic cascade also impinges upon the responding tumor microenvironment, namely tumor stromal cells. Indeed, solid tumors are composed of a multitude of stromal cell types in addition to cancerous cells. Among the stromal cell types that have been implicated in tumor promotion are endothelial cells, which comprise the blood and lymphatic circulatory systems,

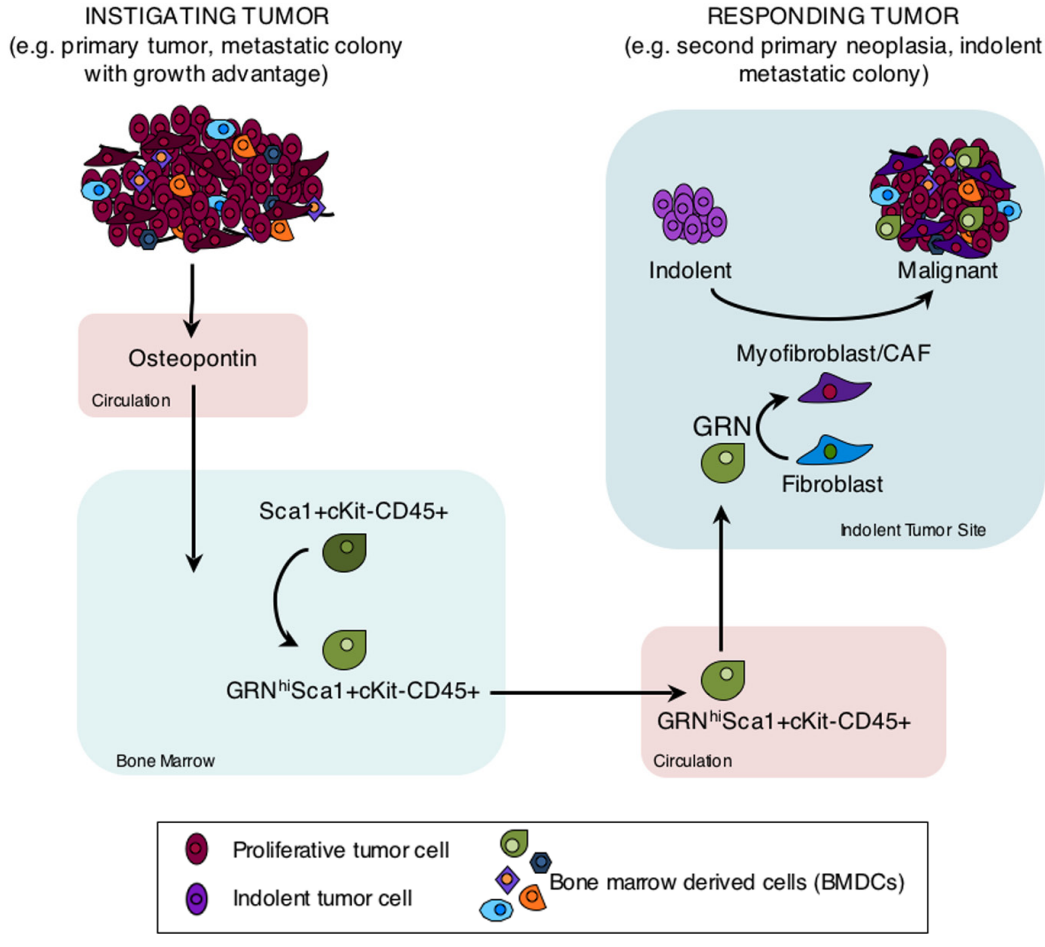

Fig. 1. Systemic instigation. *An instigating tumor (which may represent a primary tumor, residual disease, or a metastatic colony that has acquired a growth advantage) secretes factors, including osteopontin (OPN), which are necessary to activate pro-tumorigenic cells in the bone marrow. In the marrow, Sca1+/cKit- cells are stimulated to upregulate granulin (GRN) expression, before they are subsequently mobilized into the circulation and migrate to the site of an indolent tumor (which may represent a second primary lesion or a dormant metastatic colony). The GRN^{hi}Sca1+cKit- BMCs activate tumor-supportive fibroblasts to adopt features of cancer-associated fibroblasts (CAFs). These CAFs, in turn, promote the conversion of indolent tumor cells into a malignant tumor.*

pericytes, fibroblasts, and various bone marrow derived cells (BMDCs), such as macrophages, neutrophils, mast cells, myeloid cell-derived suppressor cells, and mesenchymal stem cells (Joyce and Pollard, 2009).

Fibroblasts are primarily responsible for the synthesis, deposition, and remodelling of the extracellular matrix (ECM), as well as for the production of many soluble paracrine growth factors that regulate cell proliferation, morphology, survival, and death. Historically, fibroblasts were thought to be passive participants during neoplastic development; however, several studies indicate that they exert an active role and, in combination with inflammatory cells, can promote neoplastic programming of tissues (Fleming *et al.*, 2010, Martin *et al.*, 2009, Pazolli *et al.*, 2009, Romanov *et al.*, 2001, Tlsty and Coussens, 2006, Wallace *et al.*, 2011). Ultrastructural studies, immunhistochemistry, and biochemical analysis have each contributed to the appreciation that the microenvironment is altered at critical steps during the neoplastic process (Ronnov-Jessen *et al.*, 1996). In tumors, fibroblasts have been referred to as myofibroblasts, peritumoral fibroblasts, reactive stromal cells, and carcinoma-associated fibroblasts (CAFs). They typically exhibit a higher proliferative index compared to fibroblasts in normal tissues, often express α-smooth muscle actin (αSMA), and are commonly surrounded by dense accumulations of fibrillar collagens (Ronnov-Jessen *et al.*, 1996). This phenotype — common in nearly all malignant adenocarcinomas — is termed desmoplasia and is associated with the recruitment of inflammatory cells and activation of angiogenic programs.

The pro-tumorigenic systemic environment also eventually impinges upon the tumor microenvironment. Systemic instigation, at least as defined above, is mediated, in large part, by a subpopulation of Sca-1+/cKit–/CD45+ bone marrow cells (BMCs) that are activated in response to a growing instigating breast tumor (Elkabets *et al.*, 2011). Sca-1+/cKit– cells have been identified as a subpopulation of progenitor cells that can give rise to all hematopoietic lineages, and have even been shown to give rise to the hematopoietic stem cell (Lin-/Sca1+/cKit+) (Harman *et al.*, 2008, Kumar *et al.*, 2008, Randall and Weissman, 1998, Trowbridge *et al.*, 2010, Xiao *et al.*, 2008). In mice bearing instigating tumors, these cells are rendered pro-tumorigenic in the bone marrow prior to their differentiation and mobilization into the circulation, and are induced to express high levels of the secreted glycoprotein granulin (GRN). A member of the epithelin family of growth factors, GRN is expressed by multiple cells types, including hematopoietic cells, epithelial cells, and some neurons. GRN promotes proliferation, migration, and survival and regulates inflammation, and high GRN expression in tumors has been correlated with high-grade lesions (Bateman and Bennett, 2009, Elkabets *et al.*, 2011). Consistent with a potential role for GRN in mediating stromal desmoplasia, GRN is highly expressed in wound tissues and has been demonstrated to increase the numbers of fibroblasts and capillaries that enter wounds in the early stages of healing (He *et al.*, 2003).

Once mobilized, the pro-tumorigenic GRN-expressing Sca-1+/cKit–/CD45+ BMCs travel to sites where incipient tumors reside. Here, GRN secreted from these BMCs mediates the assembly of a desmoplastic tumor stroma by stimulating fibroblast differentiation. Fibroblasts stimulated with GRN elaborate proteins that define cancer-associated fibroblasts (CAFs), including αSMA and a host of pro-inflammatory and matrix remodeling cytokines (Elkabets *et al.*, 2011, Erez *et al.*, 2010). These activated CAFs, in turn, support growth of the otherwise indolent tumors (Fig. 1). Such reactive stroma is nearly always observed in malignant adenocarcinomas and is correlated with reduced patient survival (Bissell and Radisky, 2001, Kalluri and Zeisberg, 2006, Walker, 2001). High levels of GRN expression correlate with the most malignant subtypes of breast cancer, namely triple negative (TN) and basal-like breast cancers, and predict reduced survival of breast cancer patients (Elkabets *et al.*, 2011). In fact, GRN expression also negatively correlates with the more differentiated and less invasive luminal breast cancer subtypes.

Clinical relevance of the tumor-supportive systemic environment

While the process of systemic instigation was discovered in a human tumor xenograft model, clinical data suggests that the mechanisms of instigation identified in the laboratory could be important in human disease as well. Up to 10% of breast cancer patients present with distant metastases at diagnosis, and surgery to remove the primary tumor improves patient survival (Klein, 2009, Ruiterkamp *et al.*, 2009, Ruiterkamp *et al.*, 2010). Since breast cancer deaths are most often due to metastatic disease rather than the primary tumor (Jemal *et al.*, 2008), the fact that removal of the primary tumor promotes survival of patients with metastases suggests that signals from the primary tumor may contribute to the malignancy of distant metastases. Furthermore, a small percentage of women diagnosed with breast cancer present with synchronous bilateral disease, i.e. tumors in both breasts. Such patients have significantly poorer overall survival compared to those with metachronous bilateral or unilateral tumors (Carmichael *et al.*, 2002). A large study of patients with invasive breast cancer (IBC) found a 2-3 fold increased risk of the development of a tumor in the contralateral breast (Schaapveld *et al.*, 2008), and breast cancer patients also have a high risk of developing a second primary cancer in another organ (Okamoto *et al.*, 1987). While there are many possible explanations for these clinical phenomena, these observations support the notion that signals from a primary breast tumor may promote the growth of an otherwise indolent, undetectable tumor in the contralateral breast or another organ.

In many cases, the timing with which multiple foci of recurrent disease are detected appears to be synchronized. The mathematical likelihood of finding only one lung metastasis as the first recurrent lesion 10 years after breast cancer surgery is statistically higher than finding two or more (Withers and Lee, 2006), but clinical observations and follow up studies show that most breast cancer patients present with two or more lesions at the time of first recurrence (Greenberg *et al.*, 1996, Swenerton *et al.*, 1979, Tomiak *et al.*, 1996, Withers and Lee, 2006). Furthermore, autopsy studies have shown that patients with metastatic cancer have an average of 5.6 metastases in 2-3 organs (Klein, 2009). It is unclear why metastases would appear suddenly and synchronously. Nevertheless, these findings support the idea that there may be a systemic and synchronized instigation-like process in which one metastatic colony that has spontaneously acquired a growth advantage stimulates the growth of incipient disseminated tumor cells or micrometastases at distant sites in the same cancer patient.

Many cancer patients have been found to harbor disseminated cancer cells (DTCs) in their peripheral blood and bone marrow, as well as at common sites of visceral metastasis, such as the liver

and lungs, even up to 20 years after successful treatment of the primary tumor and in the absence of clinically detectable recurrence (Aguirre-Ghiso, 2007, Fehm *et al.*, 2008, Klein, 2009, Nagrath *et al.*, 2007, Pantel *et al.*, 1999). Since only a subset of these DTCs and pre-malignancies will escape dormancy and become clinically relevant tumors or metastases, an understanding of the signals that mediate escape from dormancy is critical. Future studies along these lines stand to unify the existing experimental theories about early dissemination of metastases and the clinical observations of disease recurrence months or years after surgery and chemotherapy. Appropriate preclinical models will likely be very important tools in the search for factors that contribute to the development of disease relapse as well as for testing therapies aimed at interrupting tumor-supportive systemic processes.

Systemic instigation in mouse xenograft models

A number of immunocompromised mouse strains have been used for the study of human tumor growth though xenograft implantation, and have yielded an abundance of clinically relevant results (Al-Hajj *et al.*, 2003, Behbod *et al.*, 2009, Bos *et al.*, 2009, Kang *et al.*, 2003, Kim *et al.*, 2009, Minn *et al.*, 2005, Quintana *et al.*, 2010). The Nude mouse is one of the least immunocompromised strains. Nude mice are essentially athymic, and therefore lack mature T and B cells. However, they do have normal B and T cell precursors in the bone marrow, as well as functional NK cells and macrophages (Clarke, 1996, Liu and Hicklin, 2011). Other mouse strains commonly used for human tumor xenograft studies include SCID, NOD-SCID, and Rag-1 deficient mice. SCID mice lack mature and pre-B and T cells while retaining normal NK cells and macrophages, NOD-SCID mice lack B and T cells, NK cells, and macrophages (Clarke, 1996, Liu and Hicklin, 2011), and Rag1-deficient mice lack mature and pre- B and T cells (Mombaerts *et al.*, 1992). Interestingly, the systemic instigation process that was identified in Nude mice is significantly less efficient in NOD-SCID and Rag1-deficient mice (unpublished observations).

These observations suggest that a component of the immune system that is functional in Nude mice but not the other immunocompromised strains may be involved in the creation of a tumor-supportive macroenvironment. Indeed, recent studies have highlighted the importance of B and T cells and macrophages in tumor initiation and progression (Andreu *et al.*, 2010, de Visser *et al.*, 2005, DeNardo *et al.*, 2009, Shiao and Coussens, 2010). These elegant studies defined the immune infiltrate in developed tumors, but did not report on the presence of early hematopoietic precursor cells (e.g., homologues of the Sca1+/cKit-/CD45+ cells). The systemic instigation model indicates that tumor-supportive BMCs are active *prior* to their differentiation and mobilization into the circulation and subsequent recruitment into incipient tumors. Future studies of precursor/product relationships and the fate of hematopoietic progenitor cells at the tumor site will likely be very informative.

New era: new instigators?

The tumor-supportive systemic environment created by certain aggressively growing tumors or metastatic colonies might provide one explanation for the outgrowth of dormant disseminated tumor cells in patients. However, it is possible that other insults induce the outgrowth of otherwise dormant cells. In 1863 Virchow hypothesized that cancer originates at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, which he called “promoters”, enhance cell proliferation due to tissue injury (reviewed by (Balkwill and Mantovani, 2001)). When tissues are wounded or exposed to a chemical irritant, cell proliferation is enhanced to facilitate tissue regeneration or wound healing, thus maintaining homeostasis. Furthermore, it has been shown that individuals suffering from chronic inflammatory disorders harbor a greatly increased risk for cancer development, owing primarily to the pro-growth environment generated by activated inflammatory cells (Tlsty and Coussens, 2006).

Based on this theory, several researchers have analyzed the effects of surgery on patient outcome (Retsky *et al.*, 2008). Evaluation of disease recurrence patterns in more than 1,000 breast cancer patients demonstrated that cancer patients who did not undergo surgery showed a single peak of recurrence approximately 4-5 years following diagnosis (Demicheli *et al.*, 1994). In contrast, a bi-modal pattern, which could not be explained by a continuous tumor growth model, was observed in patients who underwent mastectomy. The recurrence pattern showed an early peak at approximately 18 months after surgery, whereas a second peak of recurrence was documented at approximately 60 months, followed by a plateau-like tail extending up to 15 years (Demicheli *et al.*, 2007). The surgical techniques by which tumors are removed have recently been shown to influence outcome. Indeed, open resection of colorectal cancer was associated with shorter disease-free interval and time to recurrence compared with laparoscopic resection (Coffey *et al.*, 2003). It has been speculated that these patterns can in part be explained by surgery-driven interruption of dormant micrometastatic breast cancer by a surge of growth factors (Coffey *et al.*, 2003, Demicheli *et al.*, 2007, Retsky *et al.*, 2008).

In agreement with these studies, metastatic models of murine mammary carcinoma indicate that hepatic surgery prior to intravenous injection of tumor cells promoted colonization in the liver of tumor cells that would otherwise not form metastatic colonies (Murthy *et al.*, 1989). Tumor burden was significantly reduced if the injection of cancer cells was delayed relative to surgery, suggesting that the acute and early wound healing processes supported tumor implantation. These findings have been echoed by studies demonstrating that certain bone marrow derived cells help to establish “pre-metastatic” niches (Hiratsuka *et al.*, 2002, Kaplan *et al.*, 2005).

In addition to surgery, wound trauma has been associated with clinical manifestations of recurrent disease (Gamatsi *et al.*, 2000, Kotzen *et al.*, 1999, Morihara *et al.*, 2007, Oosterling *et al.*, 2005). In experimental studies, burn injury resulted in a rapid mobilization of circulating endothelial precursor cells from the bone marrow, ostensibly in response to elevated levels of plasma vascular endothelial growth factor (VEGF) (Gill *et al.*, 2001). Other studies elegantly demonstrated the importance of wound-induced inflammation on the transformation of local epithelial cells (Bissell and Radisky, 2001) (Dolberg *et al.*, 1985, Sieweke *et al.*, 1990). These studies are supported by the fact that carcinomas have been known to arise in post-burn or wound scar tissue in human patients (Bowers and Young, 1960, Horton *et al.*, 1958, Flook *et al.*, 1986).

The contribution of inflammation and inflammatory cells to tumorigenesis and disease progression has been well established. During wound healing or at sites of infection, macrophages secrete

factors that recruit other circulating cells and have been shown to promote tumor malignancy and modulate response to therapy (DeNardo *et al.*, 2010, Joyce and Pollard, 2009, Wyckoff *et al.*, 2004). Carcinoma cells do not appear to be passive recipients of inflammatory cells. Instead, various carcinomas have been shown to express chemotactic factors, such as colony-stimulating factor 1 (CSF-1) to attract macrophages that express the receptor, CSF-1R (Lin and Pollard, 2007). Macrophages, in turn, promote tumor growth through secretion of growth factors, such as epidermal growth factor (EGF), which signals via the EGF receptor expressed on tumor cells (DeNardo *et al.*, 2011, Wyckoff *et al.*, 2004). Inhibition of this paracrine loop results in suppression of tumor growth and metastasis, indicating that tumor-associated macrophages are essential for tumor progression (Aharinejad *et al.*, 2004, DeNardo *et al.*, 2011, Wyckoff *et al.*, 2004). Another cytokine, the secreted glycoprotein osteopontin (OPN), has attracted considerable attention, as it plays important roles in both wound healing and establishing the tumor-supportive systemic environment (McAllister *et al.*, 2008, Pazolli *et al.*, 2009, Tuck *et al.*, 2007). In addition to these roles, OPN has pro-migratory effects on macrophages, dendritic cells and T cells within the tumor microenvironment (Buback *et al.*, 2009). During wound healing, OPN is involved in granulation tissue formation and scarring and influences fibroblast behavior (Miyazaki *et al.*, 2008, Mori *et al.*, 2008). High expression of OPN also correlates with the appearance of metaplasia after chronic inflammation (Chang *et al.*, 2011). Hence, these various physiological processes might inadvertently establish a pro-tumorigenic systemic environment that would support growth of incipient tumor cells that are poised to respond to them (Fig. 2).

Conclusions and perspectives

It has been evident for over a century that cancer is a systemic disease, and our interpretation of this concept is continually evolving. The active participation of the complex host macroenvironment in tumor progression is only beginning to be appreciated. Ultimately, the manner in which systemic cascades impinge upon the tumor microenvironment is likely to determine the fate of incipient tumor

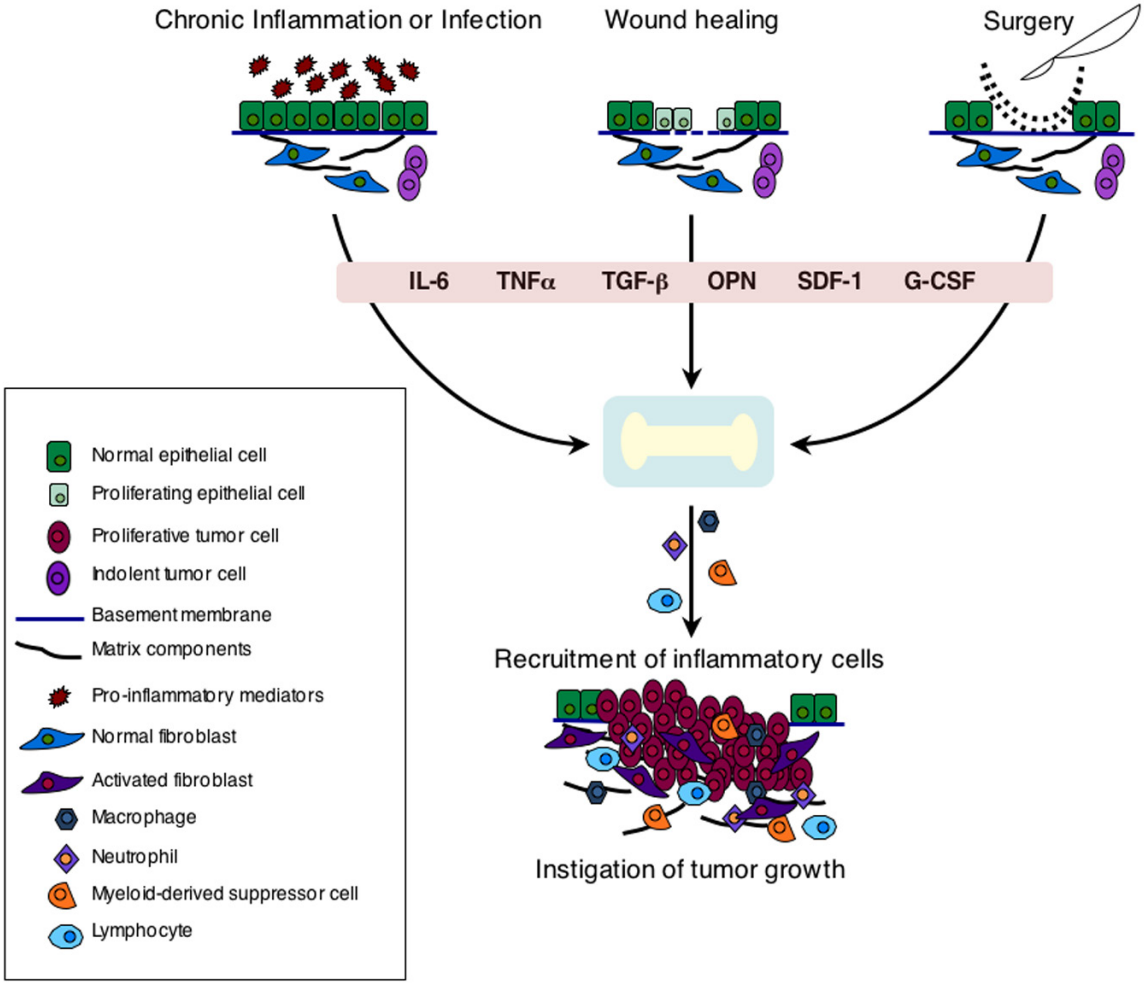

Fig. 2. Pathways that connect chronic inflammation, wound healing, and surgery with cancer. *Conditions of chronic inflammation, wound healing, or surgery produce a host of inflammatory mediators (only some of which are indicated), thereby inducing mobilization of bone marrow derived cells into the circulation. These inflammatory cells are recruited to the wounded tissue where they induce activation of fibroblasts into myofibroblasts. These processes serve to create a microenvironment that is conducive to the outgrowth of indolent tumor cells. In this figure we have omitted endothelial cells and the vasculature, obviously important microenvironmental components, as they are not specifically addressed in this review.*

cells. There are many questions that remain unanswered. For example, it remains obscure how pro-tumorigenic BMCs are activated and how they are trafficked from the bone marrow into various tumors. It is not clear what regulates the final differentiation and function of bone marrow derived and stromal cells at sites where tumors or incipient metastases reside. It is also unknown whether there are cellular and molecular components that are common to all types of pro-tumorigenic macroenvironments or whether these are specific to particular tissues and tumor types (Camp *et al.*, 2011).

Several other outstanding issues also require further clarification. For example, despite the diversity of tumor types and transforming events, are there aspects of cancer-related inflammation and/or instigation that are common to all malignancies? Would the blockade of specific pro-tumorigenic BMCs inhibit disease recurrence? Would the neoadjuvant administration of anti-inflammatory or other anti-growth factor drugs lead to a reduction of recurrence? The challenge for the future is to gain sufficient biological insight to reverse or inhibit the tumor-promoting effects of the macroenvironment, while finding ways to promote a tumor-suppressive environment.

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