Tumor Microenvironment and Differential Responses to Therapy

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Cancer evolution plays a key role in both the development of tumors and their response to therapy. Like all evolutionary processes, tumor evolution is shaped by the environment. In tumors, this consists of a complex mixture of nontransformed cell types and extracellular matrix. Chemotherapy or radiotherapy imposes further strong selective pressures on cancer cells during cancer treatment. Here, we review how different components of the tumor microenvironment can modulate the response to chemo- and radiotherapy. We further describe how therapeutic strategies directly alter the composition, or function, of the tumor microenvironment, thereby further altering the selective pressures to which cancer cells are exposed. Last, we explore the consequences of these interactions for therapy outcomes and how to exploit our increasing understanding of the tumor microenvironment for therapeutic benefit.

Solid tumors contain a complex mixture of noncancerous cell types and matrix components. Collectively, this is referred to as the tumor microenvironment or tumor stroma. The microenvironment plays a critical role in many aspects of tumorigenesis. It generates the tumor vasculature and it is highly implicated in the progression to metastasis. More recently, it has become clear that the tumor microenvironment affects the response to therapies. Further, modulating the tumor stroma may improve the efficacy of existing therapies and could present new opportunities for therapeutic targeting. In this article, we introduce the key features of the tumor microenvironment and then discuss how they influence the selective pressures on cancer cells during targeted, chemo- and radiotherapy.

COMPOSITION OF TUMOR MICROENVIRONMENT

Tumors contain various noncancerous cells including fibroblasts, vascular endothelial cells, and immune cells, including T-cells, macrophages, and neutrophils (Fig. 1) (Hanahan and Coussens 2012). In many cases, organ-specific interstitial cells are also present, for example, osteoblasts in bone tissue and astrocytes in the central nervous system. Collectively, these cells are often termed the tumor stroma and, together with factors such as the extracellular matrix, oxygen levels, and pH, they make up the tumor microenvironment. Because of space constraints, we will only briefly outline the role of stromal cells here. Endothelial cells form the

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E. Hirata and E. Sahai

Figure 1. Major components of the tumor microenvironment. Illustration of the main cellular types found within tumors alongside a table listing their main roles within the tumor.

tumor blood vessels and are critical for the delivery of oxygen, nutrients, and drugs to the tumor. Further, they provide an exit route for metabolic waste products and metastatic cancer cells (Reymond et al. 2013). Unlike normal vasculature, tumor vessels are often disorganized leading to local variations in tumor oxygenation and other environmental factors (Harney et al. 2015; Eales et al. 2016). Switching from oxidative phosphorylation to glycolysis is considered to be one of the adaptation strategies of cancer cells to survive in hypoxic conditions (Gatenby and Gillies 2004), although it also works advantageously to produce nucleic acids and nicotinamide adenine dinucleotide phosphate (NADPH) for cell proliferation (Vander Heiden et al. 2009). A by-product of this is increased lactate levels and therefore lower extracellular pH can be a feature of tumors (Damaghi et al. 2015).

Cells from both the innate and adaptive immune system are found within the tumors (Hanahan and Coussens 2012). The adaptive immune system can be capable of recognizing tumor cells as “not normal” and CD8⁺ cytotoxic T lymphocytes (CTLs) can target them for killing, a process called tumor immune-surveillance (Grivennikov et al. 2010). It is increasingly appreciated that overcoming immune surveillance is a critical part of tumorigenesis (Mittal et al. 2014) and reactivating the process by suppressing “checkpoints” that limit T-cell function is a potent anticancer strategy (Melero et al. 2015; Miller and Sadelain 2015). Innate immune cells, including macrophages and neutrophils are recruited into tumors by similar mechanisms to those that attract them to wounds. They can be both anti- and protumorigenic and cross talk extensively with endothelial cells and the innate immune system (Qian and Pollard 2010). Fibroblastic cells, including resident tissue fibroblasts, pericytes, and mesenchymal stem cells can become activated in tumors. Activated fibroblasts, termed cancer-associated fibroblasts (CAFs), produce and remodel much of the extracellular matrix within tumors (Bhowmick et al. 2004; Kalluri and Zeisberg 2006; Hanahan and Coussens 2012). This can often lead to elevated levels of tissue stiffness in tumors (Levental et al. 2009). CAFs are

generally proinvasive and proangiogenic (Madar et al. 2013), although recent evidence shows that they are not universally protumorigenic (Ozdemir et al. 2014; Rhim et al. 2014). Readers are directed to several excellent reviews describe the various components of the tumor microenvironment in detail (Joyce and Pollard 2009; Hanahan and Weinberg 2011; Hanahan and Coussens 2012; McAllister and Weinberg 2014).

To summarize a large body of work, cancer cells and stromal cells can interact in ways that may either favor or hinder tumor progression (Fig. 2). These environmental influences significantly shape tumor evolution. Cancer cells are under selective pressure to maximally exploit favorable microenvironmental conditions and overcome unfavorable ones. The former situation is exemplified by the gain of chemokine receptor expression in various carcinomas. The tumor microenvironment can often contain high levels of chemokines, such as C-X-C chemokine ligand (CXCL) 12/stromal derived factor (SDF) 1 (Orimo et al. 2005). The availability of CXCL12 then means that cancer cells expressing the relevant receptor, C-X-C motif chemokine receptor (CXCR) 4, will be at an advantage. Indeed, high levels of CXCR4 expression are associated with high CXCL12 levels in the primary tumor and metastasis to tissues with high CXCL12 levels (Zhang et al. 2013). Conversely, The gain of immune-suppressive molecules, such as programmed death ligand 1 (PD-L1), can lead to cancer cells overcoming the presence of CTLs that express PD-1 in the tumor microenvironment (Iwai et al. 2002). In addition, there is selective pressure for cancer cells to gain traits that promote the recruitment of protumorigenic stroma. This is perhaps best exemplified by the gain-of-expression of the proangiogenic ligands by cancer cells (Carmeliet and Jain 2000).

Variations in physical factors, such as tissue stiffness, matrix geometry, and electromagnetic fields are also features of the tumor microenvironment. For example, stiffened extracellular matrix prepared by CAFs enhances integrin-mediated mechanotransduction related signals, which strongly support cancer cell survival, proliferation, and invasion (Paszek et al. 2005; Butcher et al. 2009; Sulzmaier et al. 2014). Elec-

E. Hirata and E. Sahai

Figure 2. Major mechanisms by which the tumor microenvironment modulates the response to therapy. Stromal cells, including macrophages, endothelial cells, and fibroblasts, can produce growth factors, cytokines, and chemokines that locally promote cancer cell survival. Fibroblasts also play a major role in shaping the tumor extracellular matrix and this can promote prosurvival signals via integrins. The production and activation of transforming growth factor β (TGF- β) by stromal cells can lead to immune suppression that further protects cancer cells; this can be both local and systemic. In addition, the reduction in leukocyte numbers caused by cytotoxic and radiotherapy can lead to further immune suppression.

tric/magnetic fields created in the tumor microenvironment strongly affect cancer cell mitosis, which is already used in the treatment of glioblastoma (Stupp et al. 2012; Swanson et al. 2016). Thus, the tumor microenvironment contains a complex mixture of biochemical and biophysical cues that modulate cell behavior and provide the environment in which the fittest cancer cells are selected in the absence of therapy. These same factors can also modulate the strong selective pressures applied by chemo- and radiotherapy. Cancer treatments can also directly affect many of the cellular components of the microenvironment and further alter the context in which cancer evolution occurs.

CANCER-STROMA INTERPLAY UNDER CYTOTOXIC REAGENTS

The majority of cytotoxic chemotherapy agents either cause DNA damage, which is more diffi-

cult for cells that are replicating their DNA to resolve, or perturb mitosis. Although these agents can cause levels of damage and structural defects that are incompatible with cell viability and lead to rapid cell death in vitro, the situation in vivo is more nuanced. Imaging studies have revealed that the kinetics of cell death are much slower in vivo and it is more likely that cytotoxic agents trigger cell death through interaction with various “checkpoints” and engagement of the apoptotic machinery (Janssen et al. 2013). These latter processes can be affected by external cues; indeed, several studies have indicated how the tumor microenvironment can modulate responses to cytotoxic drugs (Sherman-Baust et al. 2003; Gilbert and Hermann 2010; Nakasone et al. 2012; Sun et al. 2012; Dijkgraaf et al. 2013).

The cell death caused by chemotherapeutic agents can act as a trigger for the recruitment of myeloid cells (Ruffell and Coussens 2015). This

is potentially because the dying cells generate similar signals to a wound. Indeed, the C-C motif chemokine ligand (CCL) 2/C-C motif chemokine receptor (CCR) 2 and colony-stimulating factor (CSF)1/CSF1R axes play an important role in this process (DeNardo et al. 2011; Qian et al. 2011; Hughes et al. 2015). In the longer term, cytotoxic drugs may systemically reduce leukocyte numbers or skew the diversity of leukocytes produced, because they disrupt the expansion of hematopoietic stem cells. It is unclear what effect this has on the efficacy of cancer cell killing; however, it is associated with significant side effects, including neutropenia. To counteract this, granulocyte (G)-CSF is often given to patients to boost neutrophil numbers (Bennett et al. 2013). However, recent work has suggested that boosting neutrophil numbers in this way may actually lead to more aggressive tumor phenotypes (Antonio et al. 2015; Wculek and Malanchi 2015a,b).

The presence of tumor-associated macrophages can have several consequences for the tumor. This is partly attributable to the presence of different macrophages subtypes within tumors (Mantovani et al. 2005). In reductionist coculture experiments, macrophages can reduce the sensitivity of cancer cells to paclitaxel, etoposide, and doxorubicin (Shree et al. 2011). Signal transducer and activator of transcription (STAT) 3 and downstream transcription from inflammatory modulators are required in macrophages for the protection of pancreatic ductal adenocarcinoma cells (Mitchem et al. 2013). The production of cathepsin B by macrophages is important to protect breast tumors from the effect of paclitaxel (Bruchard et al. 2013). This may be caused by cathepsin B activating the inflammasome and thereby elevating the production of a range of cytokines. Interleukin (IL)-6 is a possible mediator of this chemoprotection in both contexts. In addition to the production of growth factors and proteases, macrophages can affect cancer cell behavior through the production of exosomes, which are ~150-nm lipid-enclosed cell fragments. The exosome-mediated transfer of miR-155 from monocytes to neuroblastoma cells reduces the cancer cells' sensitivity to cisplatin (Challagundla et al.

2015). Activated "M2-like" macrophages recruited to tumors following chemotherapy can also affect other aspects of the tumor phenotype. They express CXCL12/SDF1 α , which is a promigratory cue for many cancer cells, and they produce vascular endothelial growth factor A (VEGF-A), which modulates the tumor vasculature and its leakiness (Du et al. 2008). The combination of these events may contribute to increased dissemination of tumor cells following chemotherapy. Tumor-associated macrophages are also capable of immune suppression (Doedens et al. 2010; Ruffell et al. 2014). Together, these mechanisms tend to favor cancer cell survival following chemotherapy and support the idea that targeting macrophages may enhance the ability of conventional chemotherapy to eliminate tumors.

DNA damaging agents, such as doxorubicin, will also trigger DNA damage in stromal cells. Notably, triggering DNA damage in endothelial cells leads to increased NF- κ B activity and the elevated production of numerous cytokines, including the antiapoptotic cytokine IL-6, IL-1 α , and granulocyte-macrophage (GM)-CSF (Tavora et al. 2014). These factors then help to protect tumor cells from DNA damage. DNA damaging agents can drive certain stromal cell types, notably fibroblasts, into a state of irreversible cell-cycle arrest called senescence (Krtolica et al. 2001). Interestingly, this state is associated with a characteristic secretome, rich in chemokines and growth factors, such as CCL2, VEGF, and transforming growth factor (TGF)- β , which is capable of reducing the effects of chemotherapy (Acosta et al. 2013). Mesenchymal stem cells can protect gastric cancer cells from cytotoxic therapies through producing exosomes that activate Ca²⁺/calmodulin-dependent protein kinase and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) (Ji et al. 2015). The various mechanisms highlighted here are from a variety of model systems and may not act at once; nonetheless, it is clear that there are a multitude of mechanisms by which the efficacy of cytotoxic agents can be reduced by cells within the tumor microenvironment.

Stromal cells can also modulate the efficacy of therapy by influencing drug access to the

E. Hirata and E. Sahai

tumor. The most well-studied example of this phenomenon is the tumor vasculature. The access of cytotoxic agents is typically reduced in poorly vascularized tumors, such as pancreatic ductal adenocarcinoma (Sofuni et al. 2005; Olive et al. 2009). However, the quality of the vasculature is also an important factor (Carmeliet and Jain 2011b). A high vessel density may not lead to efficient drug delivery if the vessels are poorly perfused. Further, high interstitial pressure within tumors can hamper drugs entering the tumor. In some systems, high internal pressure has been linked to stromal fibroblasts and the extracellular matrix. Interference with the activity of stromal fibroblasts can improve drug access and therapy response in preclinical models (Brown et al. 2003; Olive et al. 2009; Perentes et al. 2009).

STROMAL MODULATION OF RESPONSES TO RADIOTHERAPY

Radiotherapy is widely used to treat various types of cancers. Its primary mechanism of action is to cause DNA damage leading to cell death. Tumor targeting is largely achieved through physical methods of focusing the beam of radiation on the tumor. However, all cells within this region are likely to be affected. These effects of radiation on stromal cells have mainly been studied in the context of direct adverse reactions that might damage normal tissue/organ functions and worsen patients' quality of life. However, it is now becoming clear that the effect of radiation on stromal cells can affect therapeutic efficacy.

The most well-known microenvironmental factor to affect radiotherapy is oxygen tension (Semenza 2004). High oxygen levels enhance photon-based radiotherapy because oxygen can be converted to DNA-damaging free radicals by the radiation. As a result, a large effort has been devoted to improving tissue oxygenation concomitant with radiotherapy delivery (Barker et al. 2015). It has also been observed that vascular damage triggered by irradiation can contribute to its efficacy, possibly by reducing the availability of nutrients (Garcia-Barros et al. 2003). However, the situation is more

complex as vascular repair processes can be initiated after radiotherapy and these can promote cancer cell survival. Irradiation induces tumor vasculogenesis through bone marrow–derived cell (BMDC) recruitment that supports tumor growth (Kioi et al. 2010; Kozin et al. 2010). Further, radiation induces hypoxia-inducible factor (HIF) 1-mediated expression of VEGF and basic fibroblast growth factor (bFGF) in cancer cells, which promotes endothelial cell survival and ultimately radioresistance (Moeller et al. 2004). This can be overcome by inhibiting HIF1 function after delivery radiotherapy. If HIF1 is inhibited before radiotherapy, then it hampers tumor killing by reducing cancer cell proliferation. These studies provide an example of how radiotherapy interacts with the stroma and the importance of considering the phasing of interventions when trying to overcome radioresistance.

In addition to direct killing of tumor cells, radiotherapy can promote antitumor immunity. The efficacy of radiotherapy relies on induction of type I interferon-dependent innate and adaptive immunity (Burnette et al. 2011). This is partly via activation of tumor-associated dendritic cells that in turn support tumor-specific effector CD8⁺ T cells (Gupta et al. 2012). The trigger for the activation of the immune system following irradiation is the activation of damage-associated molecular patterns and their corresponding pattern-recognition receptors (DAMP-PRR) (Apetoh et al. 2007). This class of receptors recognizes molecules associated with tissue damage; specifically, cell damage leads to the release of high mobility group box (HMGB) 1 and this subsequently triggers toll-like receptor (TLR) 4 signaling in dendritic cells (Scaffidi et al. 2002). It is likely that interactions with the immune system following radiotherapy are responsible for the abscopal effect, which is when disseminated tumors that were not directly targeted by radiotherapy also respond (Kaminski et al. 2005; Postow et al. 2012; Siva et al. 2015).

Fibroblasts are highly responsive to radiation, and linked to this is the phenomenon of radiation fibrosis (Yarnold and Brotons 2010). Radiation can activate fibroblasts in many ways. Vascular damage can lead to the leakage of se-

rum components, such as lysophosphatidic acid (LPA), and proteases into tissue. LPA stimulates fibroblast contractility and extracellular matrix remodeling (Calvo et al. 2013). Proteases, including thrombin and plasmin, can activate TGF- β , which also promote the activation of fibroblasts (Barcellos-Hoff 1998). Once activated, fibroblasts can synthesize large amounts of collagens and organize them into dense fibrillary tissue. This abundance of matrix can promote cancer cell survival via integrin signaling. Blockade of integrin β 1 enhanced the efficacy radiotherapy in a model of small-cell lung carcinoma (Park et al. 2008; Mantoni et al. 2011). Fibroblasts can also promote radioresistance via a combination of Notch signaling and the transfer of exosomes that activate STAT1 to breast cancer cells (Boelens et al. 2014). Similar to cytotoxic drugs, radiation-induced DNA damage can drive fibroblasts into senescence and promote a secretome that includes high levels of TGF- β ligands, VEGF-A, and various cytokines (Freund et al. 2010; Acosta et al. 2013). Crucially, media conditioned by senescent fibroblasts confers radioresistance (Tsai et al. 2009). Increased levels of TGF- β are a common feature of the microenvironment following radiation, although it should be noted that fibroblasts may not be the only source of TGF- β . Increased TGF- β levels can affect the subsequent progression of residual disease, thereby undermining the long-term efficacy of the therapy (Tsai et al. 2009). Further, the immune-suppressive effects of TGF- β can prevent an effective immune response being triggered against the cancer cell debris that results from the cells killed by radiotherapy. Blockade of TGF- β signaling can enhance CD8-mediated killing of tumor cells following radiation (Vanpouille-Box et al. 2015).

MICROENVIRONMENTAL PROTECTION FROM TARGETED THERAPIES

The last 15 years have seen the emergence of therapies targeted against oncogene protein products. Most notably, kinase inhibitors of BRAF, epidermal growth factor receptor (EGFR) and its homologs, and Abl are now

used to treat tumors with oncogenic perturbation of these genes (O'Brien et al. 2003; Piccart-Gebhart et al. 2005; Romond et al. 2005; Shepherd et al. 2005; Druker et al. 2006; Mok et al. 2009; Flaherty et al. 2010; Kantarjian et al. 2010; Maemondo et al. 2010; Chapman et al. 2011). Despite the intention to target only cells containing the oncogenic mutation, it is now clear that kinase inhibitors can modulate the tumor stroma, and their efficacy is influenced by the presence or absence of certain stromal cues. Systematic analysis of the sensitivity of BRAF mutant melanoma cells to BRAF inhibition when cocultured with different stromal cells revealed that stromal fibroblasts reduce the efficacy of targeted therapy. This may, in part, be owing to their production of hepatocyte growth factor (HGF) that activates ERK/MAP kinase, signaling independently of the BRAF oncogene (Straussman et al. 2012; Wilson et al. 2012). In parallel, BRAF inhibitors can promote matrix remodeling by melanoma-associated fibroblasts. The remodeled extracellular matrix then promotes integrin- and focal adhesion kinase (FAK)-mediated signaling that also leads to BRAF-independent ERK/MAP kinase activity (Hirata et al. 2015). BRAF targeted therapies can also lead to selection for cancer cells that have increased expression of receptor tyrosine kinases in both melanoma and colon carcinoma (Nazarian et al. 2010; Diaz et al. 2012; Prahallad et al. 2012). The elevated level of these receptors renders the cancer cells more responsive to the corresponding ligands and promotes BRAF-independent ERK/MAPK. Tumor necrosis factor α (TNF- α) production by tumor-associated macrophages can also protect melanoma cells from BRAF therapies (Smith et al. 2014) and soluble factors in cerebrospinal fluid can reduce the efficacy of BRAF inhibitors. This may partially explain the poor response of brain metastases to vemurafenib (Seifert et al. 2016). Fibroblast-derived HGF has also been implicated in the resistance of EGFR mutant lung cancer to EGFR inhibitors (Wang et al. 2009). These observations illustrate the multitude of mechanisms by which blockade of oncogenic kinases can be bypassed by signals from the tumor microenvironment.

E. Hirata and E. Sahai

CONSEQUENCES OF THE MICROENVIRONMENT FOR TUMOR EVOLUTION IN RESPONSE TO THERAPY

The various complex microenvironmental changes described above have the potential to influence the evolution of the tumor following therapy. However, it is not straightforward to predict the ultimate consequence in terms of tumor progression and patient outcome. One general theme is that many microenvironmental mechanisms that counteract the efficacy of therapy will result in a larger pool of surviving cancer cells. Initially, the large majority of these cells will not be intrinsically resistant to the therapy, but the longer they persist the more likely they are to acquire a change, either genetic or epigenetic, that confers some fitness advantage for resisting the therapy. Thus, the residual pool of cancer cells can be viewed as the “raw material” on which all subsequent cancer evolution will act. The larger this pool is, the higher the probability of a favorable change occurring that will lead to the ultimate failure of the therapy. In this way, the prosurvival cues from the microenvironment enable tumor evolution. Consistent with this, blocking FAK-dependent prosurvival cues emanating from the stroma in BRAF mutant melanoma leads to both a more dramatic reduction in the tumor volume immediately after the initiation of combination therapy and a large extension in the time to ultimate therapy failure (Hirata et al. 2015).

Although the majority of cells within a tumor may not initially harbor mutations that confer therapy resistance, it is probable that a fraction of cells will contain genetic changes that make them less sensitive to the therapy being applied. For example, a low frequency of mutations conferring EGFR inhibitor resistance has been observed in lung and colorectal cancers (Inukai et al. 2006; Maheswaran et al. 2008; Diaz et al. 2012). How the microenvironment interacts with such clones is currently unclear. However, it is possible that cells containing mutations linked to resistance may not be optimally fit before therapy; indeed, if they had a strong fitness advantage they would already be dominant. It is possible that the microenvironment

may provide supportive signals as such cells evolve toward a high level of fitness (Zhao et al. 2016).

The microenvironment also influences tumor evolution by the changing milieu of growth factors and other conditions following therapy. The altered levels of cytokines produced by stromal cells following either chemo- or radiotherapy will tend to favor cancer cells expressing the corresponding receptors. Similarly, the hypoxic microenvironment that remains following radiotherapy or antiangiogenic therapy will favor cancer cells that activate HIF1 α . This can be problematic because HIF1-regulated transcriptional programs are linked to increased invasion and epithelial to mesenchymal transition (Zhao et al. 2014; El-Naggar et al. 2015).

In addition to therapy directly triggering changes in the signaling and proliferation of stromal cells, it is possible that therapies may select for different types of stromal cells. This could lead to evolution of the tumor microenvironment. Little definitive evidence exists about the evolution of stromal cells. This is largely caused by their genetic normality and the associated low mutation rate. Nonetheless, several studies have reported some genetic alterations in cancer-associated fibroblasts (Polyak et al. 2009). Some of this work has been contentious because of the difficulty of obtaining pure stromal samples and excluding cancer cells that may have undergone a profound EMT or vascular mimicry (Seftor et al. 2012). New methods involving deep sequencing of stromal populations at various time points during tumor progression and therapy should help to clarify this matter.

TARGETING THE TUMOR–STROMA INTERACTIONS TO IMPROVE THERAPEUTIC OUTCOMES

Ultimately, the goal of cancer therapies is to eliminate the transformed cells, or at least convert them into a harmless nonproliferative state. In both cases, it is important to consider the selective pressures and possibilities for cancer evolution. In the former case, the therapy either needs to be so detrimental to the cancer cells

that the probability of the appropriate permutation of genetic or epigenetic changes occurring that would lead to resistance is so low that it is highly improbable. The likelihood of a particular set of changes occurring depends on several factors, including the number of cells, their proliferation rate, and their genomic stability. As detailed above, microenvironmental factors can undermine the efficacy of chemo- and radiotherapy, thereby leading to increased cancer cell numbers following treatment. This raises the possibility that by targeting the tumor microenvironment, or signals coming from it, the ultimate rate of therapy failure can be reduced. Currently, considerable effort is being given to identify the most effective microenvironmental targets and the existing therapies with which they should be combined (Junttila and de Sauvage 2013). One approach is to perturb either the recruitment or function of stromal cells. The combination of targeting macrophages alongside conventional therapies is yielding encouraging results. The CSF1/CSF1R axis is critical for several aspects of macrophage biology. Blockade of this axis has shown promising results in several preclinical models (Mitchem et al. 2013; Pyonteck et al. 2013); notably, it can enhance the efficacy of conventional cytotoxic therapies (Paulus et al. 2006; Ruffell et al. 2014). Genetic perturbation of CCL2/CCR2 signaling, which also affects myeloid cell recruitment, improves the efficacy of doxorubicin delivery and therapeutic responses (Nakasone et al. 2012). The targeting of CAFs has also been explored using blockade of Hedgehog signaling; however, despite encouraging preclinical data the clinical results are disappointing (Amakye et al. 2013). This is likely attributable to CAFs having both pro- and antitumorigenic functions (Ozdemir et al. 2014). Most recently, immunotherapy approaches that involve blocking the immune-suppressive cytotoxic T-lymphocyte-associated protein (CTLA) 4 or PD1 signaling have yielded promising results. These improve T-cell mediated elimination of cancer cells and can lead to durable responses. (For a more thorough review of this expanding field, readers are directed to recent articles by Pardoll [2012] and Topalian et al. [2015].)

A major focus of therapies targeting the microenvironment is the modulation of tumor vasculature. Agents targeting the proangiogenic receptor tyrosine kinase VEGFR2 are now used for the treatment of several cancer types; however, their efficacy is not as great as was initially hoped. The reasons for this are complex and extensively reviewed elsewhere (Carmeliet and Jain 2011a); but it is emerging that there are some interesting consequences for tumor evolution. The hypoxic environment that is triggered by reducing the tumor vasculature favors tumor cells with activation of HIF1 α , and this is linked to greater tumor invasion. In preclinical models, this leads to increased metastasis following VEGFR2 inhibition (Paez-Ribes et al. 2009; Keunen et al. 2011). Conversely, boosting VEGFR2 signaling can improve therapeutic responses (Wong et al. 2015). These studies highlight the complex and sometimes counter-intuitive effect of targeting the tumor microenvironment. They have also given rise to the idea that optimal therapeutic benefit may be achieved by returning the vasculature, or other components of the tumor microenvironment, to a normal state. Modest perturbation of hypoxia-responsive signaling (prolyl hydroxylase domain-containing protein (PHD) 2 heterozygosity) leads to more normal blood vessels and improved therapeutic responses (Mazzone et al. 2009). Vascular promotion may also be an effective way of delivery more drugs to tumors and avoid the selection of aggressive cancer cell phenotypes in hypoxic environments (Wong et al. 2015).

Targeting the prosurvival signals emanating from the microenvironment is another obvious way to improve the efficacy of conventional therapies. Blocking integrin signaling or downstream tyrosine kinase signaling resulting from cancer cell engagement with the extracellular matrix can improve the efficacy of radiotherapy and oncogene targeted therapy (Park et al. 2006, 2008; Hirata et al. 2015). The potential for long-term disease modulation via the microenvironment has also been shown in experiments grafting malignant cells into developing tissues. Aggressive human breast cancer cells will adopt “normal” developmental fates if

E. Hirata and E. Sahai

grafted into the developing murine mammary gland (Bussard et al. 2010; Bussard and Smith 2012). Similarly, melanoma cells will follow a neural crest fate when transplanted into chick eggs (Kulesa et al. 2006) and the chick egg environment can suppress Src-driven transformation (Dolberg and Bissell 1984). The molecular pathways underpinning these observations remain opaque; however, signals from the extracellular matrix are likely to play a role as basement membrane components can greatly reduce the malignant phenotype of human breast cancer (Nguyen-Ngoc et al. 2012). Once the signaling underpinning these findings is better understood, then the possibility of therapeutically triggering them will be an appealing possibility.

In addition to mutation and proliferation rates, the rate of tumor evolution also depends on the strength of selective pressure applied and the relative fitness differences that may occur between cancer cells. This could also be exploited for therapeutic benefit, but requires an almost opposite approach. Instead of maximizing the elimination of tumor cells, the goal becomes to minimize competitive advantages between cancer cells while preventing the tumor from reaching a size that is clinically problematic. The potential applicability of this approach is illustrated by a study that modulated the level of chemotherapy such that tumors did not reduce their size, but that tumor size was simply maintained (Gatenby et al. 2009). Interestingly, this led to better disease control (Gatenby et al. 2009). It is possible that the suppression of malignancy by developmental microenvironments described above is the result of a reduced fitness differential between the transformed and normal cells.

CONCLUDING REMARKS

The examples above illustrate the different ways in which an improved understanding of the cancer microenvironment is informing ongoing efforts to improve cancer treatment. First, the elimination of the various prosurvival cues from the environment that undermine therapy will improve initial responses and limit the

possibilities for cancer cells evolving to become therapy resistant. Second, by modulating the environment and therefore the fitness differential between cancer cells, the rate and direction of tumor evolution might be controlled. The coming years should see advances in both areas and hopefully improve therapeutic outcomes.

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E. Hirata and E. Sahai

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