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Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression

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Tumors are like new organs and are made of multiple cell types and components. The tumor competes with the normal microenvironment to overcome antitumorigenic pressures. Before that battle is won, the tumor may exist within the organ unnoticed by the host, referred to as 'occult cancer'. We review how normal tissue homeostasis and architecture inhibit progression of cancer and how changes in the microenvironment can shift the balance of these signals to the procancerous state. We also include a discussion of how this information is being tailored for clinical use.

"We find ourselves at the present time in the era of molecular biology, and we are perhaps unduly influenced by the genetic code as the dominant principle in biology. Perhaps, in a decade or two from now, the dominant principle may shift to another plane, which in turn will influence our speculations about tumour causation."[1].

—Isaac Berenblum

Recently, we informed a sample of colleagues who are not oncologists ($n = 9$) that many humans harbor potentially malignant tumors in their bodies without knowing it. Several were taken aback, but one said "very interesting; so why don't we know more people with cancer?" Why not indeed?

Here we discuss research findings over the past century providing reasonable, and at times unequivocal, evidence that many people do have 'occult' tumors. Why they had not progressed to frank cancer has remained a mystery, and the body of research literature provides few answers. We suggest that the microenvironment surrounding the tumor in these cases provides tumor-suppressive signals as long as the architecture of the tissue homeostasis is essentially controlled. However, once tissue homeostasis is lost, the altered microenvironment can itself become a potent tumor promoter, as amply demonstrated in recent research. We suggest that initiation of tumors is unavoidable, but their progression to malignancy can and should be controllable.

Genetic predispositions affect how humans age, but these are not absolute; lifestyle choices can help determine how long and how well the process can be delayed. We are optimistic that in the next quarter century advances in the rapidly expanding and exciting area of study of the normal microenvironment and lifestyle choices field will lead to revolutionary improvements in all aspects of cancer biology, from understanding progression to diagnosing and treating patients.

Camouflaged: the occult cancers

The human body is comprised of approximately ten trillion cells[2]. From the moment of conception and throughout life, these cells are assailed with radiation, oxidative damage and more. Individuals' own genetic susceptibility, damage from cigarette smoke and pollution, lack of exercise, obesity and, of course, aging itself can cause many oncogenes to get activated and many tumor suppressors to be inactivated. Yet these mutated cells that, according to current dogmas, should lose control and become autonomous do not seem to form as many cancers as would be expected from the number of harmful mutations. In fact, the majority of people live cancer-free lives for decades.

How is this possible? Considering the trillions of cells in the human body and the number of possible mutations that can or do occur and the ensuing genomic instability, the ability to restrain the aberrant growth and behavior of precancerous cells is an astonishing feat of evolutionary biology.

There are studies dating back nearly a century and now being rediscovered (for example, ref. 3), or added to, suggesting that precancerous lesions, and malignant tumors themselves, may be much more prevalent within an organism than has been thought previously. In these cases, ignorance is indeed bliss. These tumors, at their very earliest stages, have so far been found only by a thorough microscopic investigation of organs, typically at autopsy. It is not known how many of these would eventually have become frank malignant tumors. This finding was first documented in the prostate by Arnold Rich in 1935 (ref. 4) (the recently reprinted paper can be found in ref. 5). Upon routine examination of random sections of autopsied prostate tissues from men who had died of unrelated causes, Rich observed frequent "small carcinomata" in the earliest stages of prostate cancer. The frequency of these frank prostate tumors was quite high; they were present in 42 of 292 (14%) prostate specimens. Because only a single microscope slide per prostate was archived and available for analysis, Rich argued that the frequency was

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likely to be higher owing to this sampling bias. In fact, according to more recent studies, in which the entire gland was thoroughly examined, the reported frequency of histologically frank tumors was indeed much higher, 34% in men in their forties[6]. Most surprising was the discovery that *in situ* carcinoma (prostatic intraepithelial neoplasia) was present in 9% of men in their twenties, and the prevalence increased considerably with age, in 27% and 34% of men in their thirties and forties, respectively[6]. Interestingly, a similar percentage of women in their forties (39%) were found to have histologic breast cancers by postmortem examination in another study[7]. And other organs are not exempt. Similar findings are emerging for thyroid, lung, pancreas and other tissues. In fact, the frequency of occurrence in the thyroid gland is so high that the presence of these lesions is regarded as a 'normal' finding[8], and the occurrence in lung tissue, albeit less prevalent, has raised concerns about the overdiagnosis of lung cancer detected by screening[9]. A high prevalence of *in situ* cancers, or genetic rearrangements associated with cancer, is also found in the pancreas and in leukocytes. Pancreatic intraepithelial neoplasias (*in situ* precursor lesions) are "remarkably common" and also are more prevalent with age, and the Philadelphia chromosome (the chromosomal fusion between the *BCR* and *ABL* genes associated with chronic myelogenous leukemia) was detected, through the use of a sensitive PCR-based strategy, in the majority (12/16) of a small sample of healthy adults[10–12].

In some cases, primary tumors are not detected in the organ of origin but discovered as metastases. For example, Patel *et al.*[13] have reported that some breast cancers present clinically as metastases to the auxiliary lymph nodes and not as palpable lumps in the breast. Even after surgical removal and careful histological examination, the primary tumor reportedly still remained undetectable in roughly half of the patients[13]. When primary tumors were discovered, they were often smaller than the metastasis found in the lymph nodes. On the basis of these and related findings, it is evident that at least indolent or occult tumors occur much more frequently than is commonly recognized but are restrained from progressing into overt cancer by processes as yet not understood. Once the mechanisms of this protective process are elucidated, it will be possible to design therapies to either prolong protection from tumor progression and/or provide brakes when protection fails. The examples cited above provide clinical evidence that once a tumor, not always a cancer (for a more informative discussion, please see ref. 14).

Master and commander: benevolence of context

Each of the 10 trillion cells in the body has the same DNA sequence; thus, the genetic information should be essentially the same from cell to cell within a single individual if other factors do not intervene to cause shuffling of the information. As single cells multiply and tissues are formed, there is splendid reciprocity: cells communicate with each other and with the extracellular matrix (ECM) dynamically via junctions and receptors, hormones and other soluble factors. It is this mutual interchange of information between cells and their surroundings that permits the functional organization of the cells into tissues and guides organogenesis during development[15]. The process is indispensable and absolutely required for multicellular life. As such, it is not surprising that the components mediating the communication between the cell and its surroundings have an ancient evolutionary history. For example, the integrins, an important family of ECM receptors, have sequence elements that predate the emergence of metazoans, and large portions of integrin sequences can be found also in prokaryotes[16,17]. In fact, the large changes in the organization of cells and tissues during the evolution of multicellular organisms have occurred concurrently with changes in the diversity of the integrin subunits[17] and surely also with the changes in composition of the ECM surrounding the tissues and organs. Communication between cells and their microenvironment occurs through a complex network of signals generated by cell-ECM and cell-cell adhesion and junctional molecules, as well as by collaboration between the epithelial, stromal and other organ-specific cell types. These ECM-molecules, together with the enzymes that remodel them, organize and sculpt tissues but also directly signal to the cells. The cells respond to both soluble and insoluble factors and in turn change their microenvironment in a fugue-like reciprocity, the end result of which is a magnificent and still somewhat mysterious integrated system that guides and allows maintenance of the differentiated state. If the microenvironment were not dominant, each cell would have its own way and the result would be either a uniform lump of similar fate or absolute chaos.

Early examples of the dominance of the microenvironment on the processes that unleash cancer were gleaned from the study of the functional consequences of exposure to carcinogenic chemicals. More than 200 years ago, Percivall Pott[18] recognized the association between soot and skin cancer, and ever since the effects of coal tar in the tumorigenic process have been studied in one form or another. What has been learned from this body of work is that the chemicals within the coal tar—for example, benzo(a)pyrene derivatives—despite being known mutagens are not by themselves efficient carcinogens for the skin. The skin microenvironment was shown in the 1940s to suppress the initiated damages caused by chemical carcinogens, in effect acting as a 'mutation/tumor suppressor'[19,20]. Thus, overcoming the protective roles of the physiological microenvironment requires 'promotion' by other toxic agents.

These studies demonstrated that, for tumors to form, at least two different insults are needed: an 'initiator', usually frank mutagens, and one or more 'tumor promoters', typically agents that cause aberrant repair and fibrosis (reviewed in ref. 21). Over time, many different chemicals and treatments have been tested for their ability to either initiate or promote cancer. In fact, wounding can serve as a highly effective promoting stimulus[22–25] (for a historical review of the correlation between wounding and cancer, see ref. 26). As researchers dig more deeply into the factors that are necessary for a tumor to become a malignant cancer, it seems that there have to be many more hits than two. Yet, for three decades most of the elegant studies on oncogenes have argued that a single hit may be all that is needed. Indeed, many of the studies with mutated tumor viruses or engineered mice can be, and have been, interpreted to support the single-hit conclusion. In particular, the seminal experiment performed by Steve Martin in the 1970s[27] used a temperature-sensitive mutant of the transforming protein of Rous sarcoma virus (RSV), pp60 Src, to show that chick embryo fibroblasts were morphologically 'transformed' at 37 °C (active Src) but not at 41 °C (inactive Src), elegantly supporting the contention that under these conditions activation of a single transforming protein was sufficient to lead to transformation. Nevertheless, when the cells at the nonpermissive temperature (inactive Src) were exposed to a tumor-promoting agent (12-O-tetradecanoylphorbol 13-acetate, TPA), they showed an exaggerated 'transformed' phenotype compared to normal chick embryo fibroblasts treated with TPA[28], raising the possibility that other factors could also contribute to the promotion of oncogenesis. In fact, reports by Francisco Duran-Reynals in the 1930s and 1940s[29] had raised the possibility that embryonic context may inhibit RSV infection from causing tumors. Whereas there was never any doubt that RSV injected into the wings of chickens produced large and malignant tumors that killed the birds[30], papers on inoculation of RSV into embryos had been dismissed as artifacts of either eggs that contain other pathogens or poor virus preparations. In the backdrop of compelling data such as those discussed above[27], the results of these older experiments in embryos[29] were deemed an artifact. Nevertheless, when David Dolberg and Mina

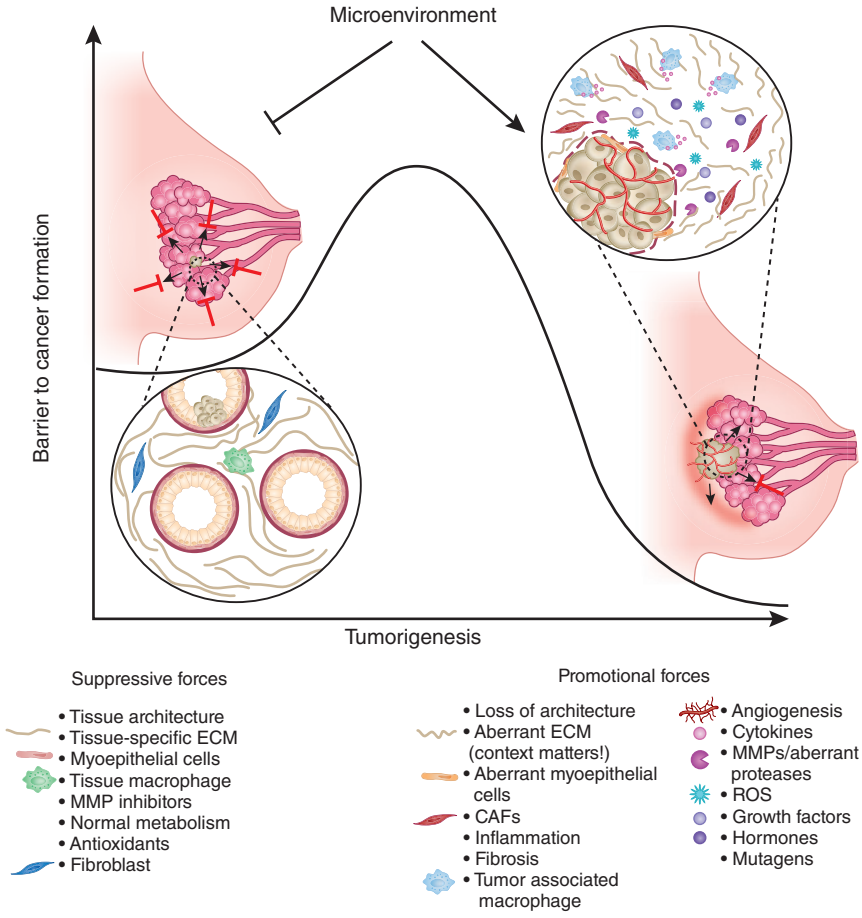

Figure 1 The normal tissue microenvironment acts as a barrier to tumorigenesis. Under conditions of normal tissue homeostasis, the microenvironment exerts suppressive forces to keep occult tumors in check (bottom left in graph). But the microenvironment can also be permissive to tumor growth, and the combination of mutagens, inflammation, growth factors and other tissue-associated promotional forces can breach the barrier to tumor formation, resulting in full-blown cancer (top right).

Bissell repeated the experiment by injecting RSV into the wings of early chick embryos that were specific pathogen free, the embryos developed normally despite the presence of the active oncogene[31]. However, when these same embryonic wings were removed from the embryo and dissociated, they quickly (overnight) showed the transformed phenotype in a culture dish[31,32]. These experiments showed clearly that the embryonic microenvironment could indeed override the ability of even potent oncogenes to cause malignant transformation. However, this inhibition was not absolute: as the embryos got closer to hatching and the microenvironment of the transduced oncogene changed, blood vessels and other tissues showed signs of aberration and disintegration[32]. Two corollary questions can be raised from these studies. First, how does the early embryo protect against the overt and active oncogene? Second, why would, even in the chicken, the RSV tumor induced by wing injection grow to kill the host with usually no additional tumors elsewhere—despite the abundance of circulating virus in the blood? Answers to these questions seem to be related to the role of the inflammatory response in oncogenic transformation even in the case of the RSV-induced tumors in the chicken[25]. In subsequent experiments it was shown that some form of injury or wounding was necessary to promote tumor formation, even in the adult chickens. We identified the active molecule in wounding responsible for 'promotion' of RSV infection to full-blown tumors to be transforming growth factor-β (TGF-β)[33], a surprising finding at the time, as this molecule has a different role in normal tissues as a suppressor of growth. This finding showed that when there is an initiating event such as a hit by a potent oncogenic virus, the physiological processes that help regulate homeostasis (such as wound healing and TGF-β) can become agents of destruction. However, everything is context dependent, as demonstrated beautifully in another study from the laboratory of Hal Moses, where in crossbreeding experiments involving the production of mice carrying transgenes encoding mouse mammary tumor virus (MMTV)–TGF-β1 and MMTV–TGF-α, they observed a marked suppression of mammary tumor formation, and the resulting hybrid mice were resistant to 7,12-dimethylbenz[a]anthracene–induced mammary tumor formation.

That context indeed matters had not been lost to some of the scientists in the field in the 1970s. There were a number of noteworthy papers by Barry Pierce[34,35] and Leroy Stevens[36,37], showing that the presence of normal cells can suppress the behavior of tumor cells. In 1974, Ralph Brinster reported a potentially important finding where 1 in 60 mice, that had been implanted with blastocysts containing teratoma cells delivered progeny with some traits of the teratoma of origin[38]. However, in two provocative papers[39,40], Beatrice Mintz and Carl Illmensee reported that although teratocarcinoma cells from an agouti mouse obtained from Stevens's laboratory[36] could form tumors in the flanks of 129/SV mice, when these cells were instead placed in the blastocyst of a pseudopregnant nonagouti (C57BL/6) mouse, the offspring showed many of the traits of the parental tumor cell and yet remained perfectly normal and tumor free. Further, they reported that the second mating with another nonagouti mouse was a complete agouti hybrid, indicating that the tumor cells were in the germ line and that the F_1 progeny were 'completely normalized'. A more recent related study, from the laboratories of Rudi Jaenisch and Lynda Chin[41], using similar techniques reported the transplantation of nuclei from leukemia, lymphoma and breast cancer cells into enucleated oocytes. These progeny showed limited plasticity in their early embryonic behavior: although all could progress to the blastocyst stage, none could produce embryonic stem cells.

Additional evidence for the role of the stromal microenvironment in influencing, or even instructing, the abnormal epithelia to become differentiated was provided by Gerry Cunha and his collaborators, among others[42,43]. More recently, engineered animals and three-dimensional culture models have made it possible to unequivocally show such plasticity and tracking in the same population of cells and the mechanisms by which microenvironmental signals, including the embryonic environment, ECM and tissue architecture, could lead to tumor cell reversion. With the advent of a versatile three-dimensional assay from the laboratories of Ole Petersen and Bissell, in which normal and malignant cells could be distinguished rapidly and robustly[44], a systematic study became possible when human breast cancer cells in three-dimensional laminin-rich gels were shown to 'revert' to a near normal phenotype[45].

That this was not selection from a heterogeneous population was shown by dissociation of the reverted colonies (in the absence of the reverting agents), where the malignant phenotype could be restored again and again[46]. Furthermore, the genome of the reverted cells was shown by comparative genomic hybridization to be no different than the mutated and malignant cells grown in two-dimensional cultures[47,48]. A series of elegant studies from the laboratory of Mary Hendrix has also shown the plasticity of aggressive melanoma cells under various conditions, including in a zebrafish[49]. Her laboratory has also developed a three-dimensional model in which melanoma cells are cultured with human embryonic stem cells (hESCs) and determined that exposure of tumor cells to different hESC matrices induced a melanocyte-like phenotype with the ability to form colonies similar to hESCs[50]. Similarly, Gil Smith recently showed that the mouse mammary gland can reprogram human embryonal carcinoma cells into cells that have phenotypes of differentiated mammary epithelial cell phenotypes[51].

In summary, the microenvironment can and does constrain the malignant phenotype in the right context, but, in the absence of evidence to the contrary, it is not necessary to insist that the tumor cells either do not have mutations or that reverted cells have lost the mutations. The phenotype is dominant over the genotype of even tumor cells; how else can one explain the occult tumors and dormancy? Indeed, how else would one explain the tissue specificity of heritable cancers, for example, BRCA1 and breast cancer, where, despite mutations in all of more than 10 trillion cells, the tumors are not only tissue specific but also formed from just one or a few cells of those tissues?

The double-edged sword: microenvironment can promote and induce cancer

We have shown above that the microenvironment can provide crucial signaling to maintain tissue architecture, inhibit cell growth and suppress or revert the malignant phenotype. It stands to reason then that the opposite must also be true: incorrect signals from the microenvironment should lead to destabilization of tissue homeostasis and initiation and promotion of normal cells to malignancy. And there is much compelling recent evidence for this statement.

As people age, the collective complex referred to as 'stroma' (fibroblasts, vasculature, immune cells and interstitial ECM) gradually changes and, over time, becomes so altered that there is accumulated damage to the epithelia as a result of miscues, even in the absence of any known genetic susceptibility. A correlation between fibrotic stroma and cancer is well established in the liver[52], and, in the breast, signs of aberrant stroma and epithelia may exist long before there is overt carcinoma. In fact, increased stromal density correlates with a higher likelihood of developing breast cancer[53–55]. Even in the absence of overt and visible signals from the stroma, the epithelial cells accumulate mutations, begin to misbehave, change shape (atypia), lose polarity and, in the breast, fill the ductal lumen to form ductal carcinoma *in situ*. Despite the myriad of mutations and changes in the genome, the majority of which are identical to those in the frank tumors[56], many of these lesions remain as ductal carcinoma *in situ*, and some even disappear. Compromising the integrity of the basement membrane or the myoepithelial layer, which is now known to be responsible for laminin 111 synthesis, allows the luminal cells to establish contacts with the stromal ECM components, such as collagen I. In turn, this leads to signals for aberrant polarity[57], upregulation of matrix metalloproteinases (MMPs) (such as MMP-9 (ref. 58)), invasion and metastasis. Meanwhile, these signals also recruit bone marrow–derived cells, for example, macrophages, neutrophils, lymphocytes and mesenchymal stem cells, to the stroma (reviewed in refs. 59,60), more associated fibroblasts become activated[61–63] and tumors generally become hypervascularized[64] **(Fig. 1)**.

In experimental animals, it has been shown that destroying the integrity of the basement membrane with MMPs[65] can lead to aberrant stroma[66] and eventually mammary tumors[67] through production of reactive oxygen species (ROS) in mitochondria and induction of genomic instability[68]. A number of elegant studies with engineered mice have shown that compromising the stroma by deleting the activity of one of the TGF-β receptors only within the fibroblasts (and possibly endothelial cells) leads to epithelial tumors, but only in the prostate and forestomach[69]. The amount of literature regarding the ability of the microenvironment and stroma to cause—as well as support—tumors is growing by leaps and bounds and will not be belabored further here; the reviews cited above lay out the literature and argue the case convincingly. The dialogue occurring between the stroma and the tumor at this early stage (to heal or not to heal?), however, is crucial for the fate of the tumor as well as the patient. A better understanding of these earlier steps could have a profound impact on the way cancer is detected, prevented and treated in the future. Such understanding would entail a more detailed characterization and understanding of the types and functions of stromal and immune cells within the tumor microenvironment.

Space constraints do not allow discussion of all the stromal components, but we will discuss the most prominent of them, fibroblasts, in further detail. Fibroblasts are responsible for production and deposition of the bulk of the ECM proteins, such as collagen I and fibronectin. They have long been recognized as constituting part of the carcinoma and are increasingly implicated as functional participants in tumor formation. We now know that fibroblasts are very heterogeneous and are also key sources of proteolytic enzymes, growth factors and cytokines.

One of the earliest implications of the heterogeneity of fibroblasts and their possible role in cancer promotion (and possibly induction) has come from the laboratory of Seth Schor and Ana Schor in the 1980s. In a seminal experiment, they isolated 77 different samples of fibroblasts from fetal, foreskin and adult normal skin and monitored their migration into three-dimensional collagen gels. The migration of transformed fibroblast cell lines was also measured. A cell density migration index (CDMI) was defined to express the rate of fibroblast migration in quantitative terms[70]. The results showed that the CDMI values of normal adult skin fibroblasts and transformed cell lines fell into two distinct, nonoverlapping groups. The CDMI values of fetal cells defined a group intermediate between normal and transformed cells, and both the bulk population and cloned fetal cells were observed to undergo a stable transition to CDMI values characteristic of adult cells around passages 50–55 in culture. What is most relevant here is that ostensibly normal (adult) skin fibroblasts obtained from the majority of individuals with carcinoma of the breast, malignant melanoma, familial polyposis coli, retinoblastoma or Wilms' tumors, all had aberrant CDMI values falling within the intermediate fetal range. Yet skin fibroblasts obtained from the majority of subjects examined with genetic or chronic diseases (for example, rheumatoid arthritis or Duchenne muscular dystrophy) showed CDMI values that fell within the normal adult range[71]. A key finding was that the fetal fibroblasts made the transition from fetal to adult behavior by ceasing to produce migration stimulatory factor, whereas the fibroblasts from subjects with cancer did not make this shift[72]. More recent studies from the Schors' laboratory have characterized migration stimulatory factor and shown it to be a truncated form of the oncofetal isoform of fibronectin[73].

Several papers published in the 1990s showed that fibroblasts can induce the tumorigenic process. A group led by Leland Chung showed that transformed fibroblasts, when co-injected with one of several nontumorigenic cell lines, will induce these cells to form tumors[74]. Cunha, Thea Tlsty and their coworkers demonstrated that cancer-associated fibroblasts (CAFs) from prostate tumors could stimulate

tumor progression of "initiated" prostate epithelial cells immortalized with the SV40 T antigen[75]. Fibroblasts treated with radiation[74,76] and senescent fibroblasts[77] were also shown to have protumorigenic activity, underscoring a relationship between aging and cancer.

Understanding the molecular mechanisms by which fibroblasts acquire an activated state (also referred to as myofibroblasts) remains an area of intense investigation. Early experiments demonstrated that culturing fibroblasts in a medium conditioned by cancer cells, or exposing them to TGF-β, leads to their activation[78] (for an early review of the roles of activated fibroblasts, see ref. 79, and for the origin of myofibroblasts, see ref. 80). Formation of myofibroblasts correlates with fibrosis and increased risk of cancer. The above and many other studies have shown that fibroblasts are not merely spectators in the tumorigenic process but often have a position at center stage, orchestrating and actively participating in the transformation process[63,69].

Pat Brown, Marc van de Vijver and their colleagues analyzed the response of 50 types of cultured fibroblasts to serum treatment using expression microarrays and identified a gene expression profile that accompanies a response referred to as the fibroblast 'core serum response'[81] or wound-response signature[82]. This signature of activated fibroblasts contains genes that have well-recognized roles in cytokine signaling, ECM remodeling, cell motility and angiogenesis. Notably, the presence of this signature in the total tumor tissues predicts poor outcome in people with breast, lung and gastric cancers remarkably well[81,82]. *In situ* immunostaining of the tumor sections used in these studies revealed that a large number of the proteins encoded by genes identified in the signature are indeed expressed by the embedded stromal cells[80], clearly indicating that the CAFs within these tumors are contributing to an expression profile that has a striking prognostic value. Similarly, when stromal cells were isolated directly from breast tumors by laser-capture microdissection and their expression patterns analyzed in a similar fashion, the resulting gene expression pattern was also able to predict clinical outcome[83].

In the last decade, much has been published on the mechanisms by which the microenvironment can promote and even induce tumors. It is intriguing that the same mechanisms that have been discovered to induce genomic instability, and eventual transformation in epithelial cells using oncogenic viruses or chemicals in cultured cells, are also induced *in vivo* by microenvironmental signals via the stroma or the immune cells. Oxidative damage seems to be a governing factor induced by hypoxia-inducible factor-1α–mediated stromal-derived factor (also known as CXCL12) signaling[84,85], MMPs[68] or activated macrophages[86] and provides another link between the inflammatory processes and stromal activation. Given that the microenvironment in general, and stromal components in particular, are now recognized as important determinants of tumor formation, a more thorough understanding of their functional role in tumorigenesis would help translating the knowledge to the clinic.

Tumors as organs: to heal "the wound that never heals"

So far, we have outlined the evidence for the existence of occult tumors, speculated that the microenvironment most probably is the stabilizing influence behind their quiescent status and provided some historical perspective and background on the now generally accepted role of the microenvironment in the maintenance of tissue specificity and organ structure and in progression to malignancy. We set out to convey in two words that 'context matters'!

In a previous review, written almost a decade ago[87], we argued that tumors evolve from an organ and retain a memory of that organ; however, once they liberate themselves from the constraints of the normal tissue microenvironment and lose the organ-specific structure as they form bizarre masses, they evolve into 'tumor organs', literally new 'evolutionary forms'. The time course of their progression is clearly not at the evolutionary scale but is extremely rapid, such that, at any given time, the tumor organ form and function will change depending on the input from the host, the microenvironment of the tumor and the genetic and epigenetic changes within the tumor cells. We referred to tumors as entities that constantly redefine themselves by their ever-changing context[87]. This raises the following conundrum: if both the tumor microenvironment and the tumors themselves are dynamic and coevolving, can the tumor organ ever be targeted successfully? The fact that we have not made more progress in curing glandular tumors may be a reflection of this conundrum. However, because the field has begun to embrace the crucial role of the tumor microenvironment in guiding tumor behavior, can it now gain enough knowledge about the tumor organ, which is indeed a "caricature"[88,89] of the actual organ from which it was derived, so that we can increase the odds for success[90]? Could we indeed rephrase the famous quotation by Dvorak on tumors—perhaps it is possible to "heal the wound that never heals"[91]? We argue that we can—and must.

The involvement of tumor stroma as a regulator of tumor fate. The existence of a reactive stroma has been known for a long time. But it is now known that the tumor need not always come first, as we discussed in the preceding sections. However, once the tumor is formed, regardless of the mechanism, it not only modifies the stroma drastically but also initiates an inflammatory reaction and complex immune response. The former either already exists before the tumor is formed and, at least partially, is responsible for its progression, or the inflammatory cells are mobilized in response to signals emanating from the tumor microenvironment. Either way, these cells eventually end up contributing to the damage, partly because the immune system becomes co-opted by the tumor and other stromal cells (much the same way that the normal regulatory tissue–specific pathways are co-opted by the tumor organ)[87,92,93]. Indeed, the microenvironment can be used as a window into the tumor's past and future, and we have discussed above the clinical prognostic potential of microarray expression signatures derived from fibroblasts and microdissected tumor-derived stroma[81–83]. Rebecca Fitzgerald's group has found that a gene signature derived from microdissected stroma from tissues of various stages of esophageal adenocarcinoma is informative in separating the stages of clinical progression[94]. This group identified TGF-β and inflammatory pathways as major components of malignant progression, which is in agreement with the long-held theory that inflammation was driving the progression of Barrett's metaplasia to adenocarcinoma.

In addition to the prognostic information, the predictive information of whether or not a patient will respond to therapy can also be obtained from stromal-derived gene expression signatures. With the hypothesis that the tumor microenvironment influences the response to therapy—an aspect that is not accounted for in gene expression signatures derived from cultured cells—Mauro Delorenzi's group developed a unique analytical approach to decouple the expression signals from mixtures of tumor and stromal cells. With this strategy, they identified a stromal signature where increased expression of stromal genes predicted patient response to neoadjuvant treatment with 5-fluorouracil, epirubicin and cyclophosphamide[95].

Remarkably, the activation or repression of specific genes or proteins within stromal cells has also been correlated with clinical outcome. The most dramatic recent example of the latter comes from studies of caveolin-1 (Cav-1), which is the principal component of caveolar membranes and is involved in transmembrane transport and signal transduction. Both epithelial and stromal cells express Cav-1. In 1995, Michael Lisanti's group recognized that Cav-1 expression becomes attenuated during malignant transformation[96]. To decipher whether Cav-1 was an

active participant or merely a bystander, they used xenograft models and found that MMTV-PyMT tumor cells behaved differently in Cav-1–knockout than in wild-type mice. Growth of the tumors was significantly enhanced in the former[97]. In two recent independent studies, both the Lisanti group and Robin Anderson's group, using tissue microarrays of breast tumors from humans, have shown that the expression of Cav-1 is an independent predictor of increased patient survival, but only when present in the stroma[98,99]. Contrary to what was believed initially, there was no correlation between clinical parameters and Cav-1 expression in the epithelium in both of these studies.

Similar conclusions have been made for the aberrant expression within the stroma of platelet-derived growth factor-β receptor (PDGF-βR)[100] and lysyl oxidase–like-2 (LOXL2)[101]. In the case of PDGF-βR, increased expression occurred in both fibroblasts and endothelial cells of colon and prostate tumors, and this was found to correlate with negative prognostic markers as well as with decreased survival[100]. Within lung and liver specimens, LOXL2, an enzyme responsible for cross-linking collagen and elastase, was also found to be overexpressed in the tumor stroma[101]. Inhibition of LOXL2 with an inhibitory monoclonal antibody in a xenograft tumor model reduced breast tumor growth and the amount of cross-linked collagen, fibrosis, and cytokine and TGF-β signaling.

Drug discovery. Historically, drug development for cancer therapy has relied heavily on high-throughput screens, using proliferation on plastic culture dishes as an endpoint, for the identification of 'lead compounds' (for discussion, see ref. 102). These methods have provided an overwhelming number of possible targets. Despite the marked efficacy of these compounds in two-dimensional culture assays and even animal models, most have been ineffective at best, and at times even harmful, or they have added only months, instead of years, to the lives of patients with metastatic cancer. Even those drugs that seem to work more effectively increase the life expectancy of patients by two to three years. This is a marked improvement, but, judged against the spectrum of available therapies and the funding spent on development and the incorporation of new agents into clinical practice over the past decade, one would have hoped for more[103]. An unfortunate reality is that, in most cases, the clinical approach for patients with metastatic cancer is palliative instead of curative, an aspect of cancer treatment that the community must work to change.

Given knowledge of the interactions among the different pathways within the tumor organs, as well as the various cell types comprising the tumor and its microenvironment, a goal should be to incorporate this knowledge into organ-specific and physiological human culture models (both nonmalignant and malignant) together with better animal models of human cancers for drug testing. The tumor organ, just like normal organs, has a three-dimensional architecture, and integration of signals and signaling pathways are very different between two dimensions and three dimensions, even for tumor cells (for example, see ref. 104). Tumors are composed of multiple cell types capable of supporting the malignant cells through the complex network of interactions discussed above[59,63,64,105,106]. These types of interactions are simply not recapitulated in two-dimensional cultures, and there is an increasing body of literature suggesting that response to therapeutic agents is also quite different for cells cultured in two dimensions versus three dimensions[107–109]. Studies of this type have suggested the cellular response to drugs used in the clinic is, not surprisingly, context dependent[108,110,111]. It is known also that a cell's sensitivity to radiation is modified when the cells are in three-dimensional cultures, findings that can be recapitulated in mice, although not yet in orthotopic models[112,113]. Furthermore, several recent studies in animal models have shown that concurrent administration of drugs that modify the microenvironment can facilitate an adjuvant response with other chemotherapeutics. Examples include the administration of the experimental hedgehog inhibitor IPI-926, which depletes tumor-associated stromal tissue in a mouse model of pancreatic ductal adenocarcinoma[114], or the intravenous administration of a pegylated variant of hyaluronidase (PEGPH20) into a prostate xenograft model[115], a therapy that is now in phase 1 clinical trials. From these and related studies, it has become clear that targeting the cells and components of the microenvironment is likely to provide profound clinical benefits. Furthermore, the scientific community should support the development of more robust and physiologically relevant assays and possibly animal models that are closer to humans evolutionarily for testing drugs. The three-dimensional studies in the last two decades have shown that cells simply behave differently when they assume structures that are closer to organs *in vivo*. This can be the result of a number of factors, such as the physical change in the cell structure (cells in the body do not stretch out as they do on plastic dishes), sensation of tensile forces[116] or integration of the signals derived from interaction among the numerous components in the ECM, for example, laminin and collagen[117,118]. Researchers need to develop more models that permit reciprocal interchange not only between the cell and the ECM but with other cell types as well. This is now achieved by culturing epithelial cells with only one other cell type, most frequently fibroblasts[119]. However, it is also being extended to include a variety of cell types that would model the tissue microenvironment much more closely than the three-dimensional models used currently (for examples see refs. 57,120), although much more remains to be designed.

What we have not discussed. This review has not covered some of the most recent and exciting aspects of microenvironmental control. We have not touched on the rapidly evolving fields of the bone marrow–derived stem cells and the premetastatic niche[121] or the role of exosomes[122], and we have barely discussed the extensive new work in the role of force and tension[116], among other topics. These are all highly relevant to the topic of this review and each deserves to be the subject of additional reviews.

Recent trials with relevance to microenvironmental therapies. We have summarized in **Table 1** our knowledge of therapies and clinical trials that touch on the role of the microenvironment in various types of cancers. Some of these therapies have already been approved by the US Food and Drug Administration (FDA) for the treatment of several cancers; for example, the angiogenesis inhibitor bevacizumab (Avastin). Others have been approved for conditions once seemingly unrelated to cancer but are now being tested in the oncology arena. For instance, the anti-inflammatory agent onsenal (Celebra) was originally approved by the FDA in 1998 for the treatment of osteoarthritis, but it is now indicated also in the treatment of familial adenomatous polyposis and is in phase 2 clinical trials for both prostate and pancreatic cancer for its reputed ability to suppress blood vessel growth. Finally, there are other drugs, the majority of which are the multikinase inhibitors, which inhibit tumor cell growth pathways (for example, BRAF, Bcr-Abl and c-Kit) and also signaling from the microenvironment (for example, vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, VEGFR-3, PDGFR and colony-stimulating factor-1 receptor). Over time, and with more complex culture models, scientists may be able to better discriminate amongst the effects of these pathways and treatments on the tumor cell, the microenvironment or both to determine the relative contribution of each to any given clinical response. Because neither of us is a physician or involved in clinical trials, we have assembled this information without prejudice, but also without intimate knowledge of pros and cons of these treatments. We have assembled **Table 1** and **Supplementary Table 1** from primary and review articles, websites maintained by the US National Institutes of Health (http://pubchem.ncbi.nlm.nih.gov/), the FDA (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm)

Table 1 Treating the tumor microenvironment: selected examples

Common name (trade name)	Company	Drug information
Endostatin (Endostar)	Simcere	Endostatin is a 20-kDa peptide fragment derived from the extracellular matrix protein collagen XVII[125]. Endostatin has a potent effect on endothelial cell proliferation and angiogenesis. It is currently used therapeutically (and in clinical studies) only in China under the trade name Endostar.
Bevacizumab (Avastin)	Genentech	In 2004, Avastin became the first FDA-approved angiogenesis inhibitor. It is a humanized monoclonal antibody with specific affinity for VEGF-A, thus inhibiting the signaling between the tumor and endothelial cells in the microenvironment[126].
Sorafenib, BAY 43-9006 (Nexavar)	Bayer	Approved by the FDA in 2005, sorafenib is a small-molecule kinase inhibitor that inhibits many intracellular and extracellular kinases. Most affected are Raf kinase, VEGFR and PDGFR, thus resulting in multiple effects, such as reduced tumor growth and angiogenesis[127].
MK-2461	Merck	MK-2461 is a small-molecule inhibitor of c-MET kinase, the receptor of the stromal-derived hepatocyte growth factor. c-MET activation has proliferative and antiapoptotic effects in the tumor cells but also stimulates endothelial cell–dependent angiogenesis[128].
Zoledronate (Zometa)	Novartis	Zoledronate belongs to the bisphosphonate class of drugs. It is a small-molecule pyrophosphate analog that binds hydroxyapatite crystals and inhibits bone resorption by osteoclasts. It also inhibits the differentiation of myeloid cells, thus tumor-associated macrophages are also affected[129,130].
Denosumab (Xgeva)	Amgen	Approved by the FDA in June of 2010, denosumab is a human antibody that binds human receptor activator of nuclear factor-κB ligand (RANKL). RANKL regulates osteoclastogenesis and is involved in pathways regulating osteoclastogenesis, tumor cell metastasis to bone and endothelial cell proliferation and apoptosis[131,132].
Anastrazole (Armidex)	Novartis	Anastrazole is a third-generation inhibitor of aromatase, a cytochrome p450 complex present in the stromal fibroblasts. Aromatase catalyzes the conversion of androgens to estrogens, and the inhibitors are approved for the treatment of breast cancer in postmenopausal women.
AMD070	Genzyme	Currently in clinical trials, AMD070 belongs to a class of drugs that inhibit CXCR4. CXCR4 is specific for stromal-derived factor-1 ligand, which is predominantly expressed by fibroblasts and pericytes[133].
DX2400	Dyax	In development, DX2400 is a new generation of MMP inhibitor, a human monoclonal antibody specific for MMP-14. Previous broad-spectrum MMP inhibitors were generally plagued by a lack of efficacy, and the majority of drug makers have since invested in other targets. However, since the end of the previous trials, much has been learned about MMPs, notably the need for drug specificity, as some MMPs are regarded as being protective, and others not. Thus, these newer inhibitors are being designed with specificity in mind[134].
MK0822	Merck	MK0822 is an inhibitor of cathepsin K, a secreted protease involved in bone resorption. Similar to bisphosphonates, inhibitors of cathepsin K proteases may protect against bone loss induced by metastatic tumor cells[135]. Cathepsin inhibitors may be useful in other contexts, as well. In a preclinical animal model of pancreatic cancer, administration of a pancathepsin inhibitor, JPM-OEt, with cyclophosphamide led to a marked reduction in tumor burden[136].
IPI-926	Infinity Pharmaceuticals	IPI-926 is a small-molecule inhibitor of the hedgehog pathway and is currently in phase 2 clinical trials. In a preclinical mouse model, inhibition of hedgehog signaling led to depletion of tumor-associated stromal tissue and enhanced delivery of gemcitibine.
TGF-β2 AP12009 (Trabedersen)	Antisense Pharma	Trabedersen is an antisense oligodeoxynucleotide with specificity for TGF-β2. It is currently in phase 1, 2 and 3 clinical trials and is being developed for the treatment of tumors that frequently express high levels of TGF-β2 (pancreatic carcinoma, melanoma and gliomas). Reductions in TGF-β2 in the tumor are likely to be profound, affecting both tumor and stromal cells (tumor cell growth, angiogenesis and immune response).
Celecoxib (Celebra)	Pfizer	Celecoxib is a specific inhibitor of cyclooxygenase-2 (COX-2). COX-2 is present in both tumor and associated stromal cells, and inhibitors of COX-2 can influence apoptosis, cell migration, proliferation and angiogenesis. Other anti-inflammatory drugs are also being tested clinically for their ability to alter the protumorigenic microenvironment in chronic inflammation[137].
AVE1642	ImmunoGen/ Sanofi-Aventis	AVE1642, a humanized monoclonal antibody, is a specific antagonist of the insulin-like growth factor-1 receptor (IGF-1R). IGF-1 derived from bone marrow stroma promotes survival and growth of multiple myeloma cells. IGF-1R signaling also contributes to angiogenesis via its influence on hypoxia-inducible factor-1α and VEGF expression[138].
BGJ398	Novartis	Currently in clinical trials, BGJ398 is a small-molecule inhibitor of fibroblast growth factor receptors (FGFRs). The ligands of these receptors, FGFs, are expressed by the activated fibroblasts of tumor stroma and have a protumorigenic effect.
Bortezomib, PS-341 (Velcade)	Millenium Pharmaceuticals	Bortezomib is an inhibitor of the 26S proteasome complex and is indicated for the treatment of relapsed multiple myeloma and mantle cell lymphoma. In addition to directly inhibiting the tumor cells, bortezomib interferes with multiple myeloma tumor and bone marrow stromal cell interactions, inhibiting cytokine signaling and angiogenesis[139,140].
PG545	Progen	Currently being tested in a phase 1 clinical trial, PG545 is a heparan sulfate mimetic, designed to inhibit heparanase activity. Heparanase inhibitors prevent ECM remodeling and release of sequestered growth factors tethered to the heparan sulfate proteoglycans located near the surface of cells, thus affecting cell growth, metastasis and angiogenesis.
PEGPH20	Halozyme	PEGPH20 is a covalently modified form of hyaluronidase, which catalyzes the degradation of the extracellular matrix component hyaluronan. In preclinical animal models, PEGPH20 led to drastic reductions of the tumor interstitial fluid pressure, subsequently enhancing the delivery of coadministered drugs. PEGPH20 is currently in phase 1 clinical trials[115].

and the websites maintained by the individual drug manufacturers. We apologize to the many scientists who have worked tirelessly to develop useful drugs if we have not succeeded in listing all relevant drugs. In the context of cancer therapy, targeting the microenvironment is still a relatively young field. Yet, as the extent of these tables indicates, examples abound.

It is our sincere hope that the scientific and clinical community put the interests of people with cancer above all other considerations and make a massive effort to discover effective combinatorial approaches that target both the tumor and its microenvironment—of course with the expectation that the side effects would not exceed, or would be even less than, those for single compound therapy. We believe the time has come to start treating cancer as a disease of organs. We also contend that it is time to start exposing young scientists to the wonders of the microenvironment in basic biology and medical texts. Everyone appreciates that DNA is central to all things, but, as we have argued before, "the sequence of our genes are like the keys on the piano; it is the context that makes the music"[123]. Cancer biologists realized the importance of context more than 100 years ago[124], probably as a result of the unavailability of sophisticated tools to probe cancer cells at the genetic level. Now that researchers know so much about genes and have also rediscovered the importance of the microenvironment, they need to make sure the twain remain acquainted!

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COMPETING FINANCIAL INTERESTS

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