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Tumor heterogeneity and its implication for drug delivery

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ABSTRACT

Evidence continues to accumulate that patient tumors contain heterogeneous cell populations, each of which may contribute differently in extent and mechanism to the progression of malignancy. However, the field of tumor drug delivery research, while continually presenting new and innovative approaches, in many ways continues to operate on the premise that essentially all tumor cells are identical. In some in vivo models, xenograft tumors using cell lines may actually be comparatively homogeneous, and thus result in overly encouraging results when a particular drug or delivery system is reported to successfully treat tumors in mice. It is well known, however, that many drugs that show success in preclinical studies will fail in clinical trials. Tumor heterogeneity is possibly one of the most significant factors that most treatment methods fail to address sufficiently. While a particular drug may exhibit initial success, the eventual relapse of tumor growth is due in many cases to subpopulations of cells that are either not affected by the drug mechanism, possess or acquire a greater drug resistance, or have a localized condition in their microenvironment that enables them to evade or withstand the drug. These various subpopulations may include cancer stem cells, mutated clonal variants, and tumor-associated stromal cells, as well as cells experiencing a spatially different condition such as hypoxia within a diffusion-limited tumor region. This review briefly discusses some of the many aspects of tumor heterogeneity and their potential implications for future drug design and delivery methods.

1. Introduction

Cancer is becoming more recognized not as a single disease, but as many, each with varying causes, prognoses, and appropriate treatments. This diversity is apparent not only across different types of cancer, but now it is also being recognized within cancers of the same tissue. Furthermore, it is now known that cancer cells within the same tumor are heterogeneous in many aspects. The heterogeneity is seen across many cell properties, including morphology or phenotypic expression, exhibition of inherent or acquired drug resistance, and capacity for initiating new tumor growth. The reasons for this extensive diversity are not fully understood. It may be a simple result of the random fluctuation of protein expression levels. However, the thought that cancer cells are all essentially identical with only natural variability accounting for differences among them is an old view, which is being replaced with a new understanding that multiple factors are responsible for the regulation and progression of tumor cell growth and differentiation. Just as an organ in the body is considered to be more than just a mass of similar cells, a tumor can also be considered in some ways to be a new, independent organ acting within the host [1]. Organs have a variety of cells at unique stages of differentiation, as well as stromal cells that support the organization of the tissue and the interaction with the rest of the body. Organs can also have complex spatial organizations that support niches where individual cells maintain specialized functions accompanied with specific supporting extracellular matrices facilitating those functions. Evidence now suggests that similar complexity exists for interactions of individual tumor cells among themselves and with the host [2–5].

Less clear, however, are the mechanisms by which tumors deviate from the integrated cooperation of an organ with the rest of the body. Clearly, tumor cells override signals that restrict unbridled cell proliferation. Some tumor cells evade apoptotic death signals or immune signals that would flag malignant cells for removal. However, they may also exploit legitimate and normally highly regulated pathways that can aid them in their survival and expansion. These may include innate differentiation and proliferation hierarchies, paracrine signaling relationships critical during embryonic development, or inflammatory signaling normally helpful in wound healing [5]. If these natural functions are mandatory for the tumor, it is not clear if the disease is continually reliant upon them or if they are only essential for initial transformation. Furthermore, differences in tumor behavior tend to evolve over time, and of course will vary from patient to patient. All of these suggest that each cancer is different and even each cell in a neoplasm can differ significantly. Here, we briefly discuss some of the likely drivers of tumor heterogeneity and propose that

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future therapy development and drug targeting must account for this heterogeneity to become effective.

2. Cancer cell heterogeneity

As the technical possibilities for evaluating clinical tumors continue to increase, so too is the evidence that cancer tissue is heterogeneous at both the intratumoral and intertumoral level. Within diagnosed cancers of a specific organ or tissue, it has become apparent that multiple neoplastic diseases can occur within the same site, but are very different in terms of morphology, progression, and drug sensitivity. This is exemplified by the multiple clinical classifications for breast cancer. Currently, breast cancer is categorized in part by the presence of certain receptors for estrogen, progesterone, or epidermal growth factor, resulting in at least five possible sub-type diagnoses: luminal A, luminal B, Human Epidermal growth factor Receptor 2 (HER-2) positive, Claudin-low, or basal-like breast cancer [6]. Each of these may warrant a different therapeutic regime, but it is becoming clear that further stratification may be necessary for improved treatment success [7]. Trastuzumab, an antibody drug developed for HER-2 positive breast cancer, is highly effective for some patients but not for others in this group. Variations in this success may depend on correct identification of other chromosomal alterations that may exist within this cancer type [8].

It is not surprising that analysis of tumor types between different patients would result in the identification of different drivers of cancer. However, tumor tissue within the same patient can also exhibit significant diversity. Human ovarian cancer cells can exhibit heterogeneity in their cellular drug resistance and the expression of stem-like phenotypes according to their spatial location within the tumor [9]. Liu et al. report the use of antibody-conjugated quantum dots to simultaneously visualize the expression of four different cellular markers on fixed biopsies of human prostate tumors. The multiplexed resolution was able to discern a heterogeneous distribution of premalignant and malignant cells located within glands that would likely appear to be benign under traditional histological evaluation [10]. Expanding beyond the primary tumor, heterogeneity is observed between metastatic growths and original tumor within the same patient. Ding et al. published an account of metastasized basal-like breast cancer from a single patient. They collected samples from the primary tumor, peripheral blood, a cerebellar metastasis, and a xenograft culture of the primary tumor, and then proceeded to sequence the genomes of each sample to find any differences [11]. A small set of mutations were noted between samples from these different sources, although in this case a majority of the mutations observed were also associated with the primary tumor. However, the tissues in the xenograft and metastasis were highly enriched for certain mutant allele frequencies, suggesting that certain subpopulations were more selected in the new environments. These findings suggest that the patterns of heterogeneity within the primary tumor may not match those found in metastatic growths [11]. In another analysis of samples from a single patient, differences among separate metastatic foci of the same melanoma patient were shown to exhibit heterogeneous morphology and surface antigen expression, suggesting new metastatic regions are also heterogeneous from each other [12]. Taken together, these data suggest that the primary tumor and each metastatic lesion might each be most effectively treated with a uniquely catered therapy regime.

While cell heterogeneity is frequently observed in tumors, there is a debate as to the source of this variability. Evidence has suggested such heterogeneity may be a product of either hierarchical or stochastic models. Here we briefly present some of the prominent viewpoints and discuss implications for targeting cell heterogeneity under such models.

2.1. Cancer stem cell theory and heterogeneity

The cancer stem cell hypothesis has gained renewed traction in recent years, although it still remains controversial as the acceptable model for tumor initiation and progression [13]. This theory asserts that tumors are initiated similarly to a developing tissue or a healing wound, driven by stem cells that give rise to proliferative and multi-differentiated progeny, while maintaining a smaller subset of progeny that retain a more quiescent and multipotent state. These so-called tumor initiating cells are also known as cancer stem cells. There is evidence in many blood cancers and in some solid tumors that there are subpopulations of cells that can initiate new tumor growth and result in a hierarchical progression of differentiation pathways [3,14]. While these side populations may be enriched with tumor initiating cells, it is not likely that all cells have equal or sufficient capacity to initiate new tumor growth [15]. The cells have been identified by cell surface markers as well as other properties, such as by dye exclusion or ALDEFLOUR assays [16]. While some interpreted this to suggest that cancer arises from a stem cell dysfunction, it is not certain that tumor originating cells must begin as normal stem cells. However, once obtaining the properties of a cancer stem cell, the premise of this theory holds that tumor initiating cells will drive tumor progression and cell differentiation in a hierarchical manner similar to other stem cell patterns, with most of the progeny entering a proliferative and differentiated state and no longer possessing stem-like capacity [13].

The cancer stem cell theory poses a significant challenge to therapeutic drug treatment. While traditional drugs have targeted the proliferation of bulk tumor cells, it is thought that the less proliferative cancer stem cells are more drug-resistant due in part to their more quiescent nature and also perhaps to enhanced mechanisms of drug exclusion [17,18]. If correct, the stem cell model poses a huge challenge to the idea of preventing tumor recurrence. If inherently drug resistant tumor initiating cells are responsible for tumor growth and progression, then blunt therapies targeting only bulk cells are likely to miss the drivers of tumor growth, leading to a likely relapse with a more aggressive progeny. On the other hand, the potential positive side of this theory is that if cancer stem cells are the engines of tumor growth and malignancy, they present a narrowly defined target for new therapeutics, giving hope for a definitive cure for cancer.

2.2. Stochastic theory and heterogeneity

The cancer stem cell theory proposes that tumor initiating cells and non-tumor initiating cells are genetically identical but exhibit their differences by epigenetic regulation. However, the stochastic model of tumor progression is based on continual genetic mutation that supports the emergence of new clonal populations evolutionarily favored to thrive in the existing environmental conditions. This would suggest that most of the cells within a clonal population would have similar tumorigenic potential, although subject to stochastic probability of actually forming a new tumor. Under this theory, heterogeneity found within a tumor would result from the existence of multiple clonal subpopulations that are maintaining sufficient viability. It is possible, however, that if one particular clonal population arises to dominate over all other populations, then the bulk of tumor cells may remain mostly homogeneous for a time until new clonal variants develop with competitive viability.

To effectively treat cancer under this model, it would be necessary to try to kill all transformed clonal populations. Furthermore, this model supports the evolutionary concept that the introduction of a therapy (or any change of condition) could select for new dominant populations. Thus, it would be necessary to devise a treatment regime capable of adapting to altered population growth rates or to try to exploit foreseen vulnerabilities caused by this phenomenon. This is in significant contrast to the cancer stem cell theory where it is really

only crucial to target the cancer stem cells with drugs as the remaining population is not expected to contribute to sufficient tumor progression to result in mortality.

2.3. Reconciliation of two theories

It is not our intention to resolve the debate between the hierarchical and stochastic models of tumor progression, but merely to reflect on how either model would influence the proper approach for effective tumor treatment. As it is quite possible that evidence will continue to mount for each theory, it is noteworthy that both hypotheses suggest that significant heterogeneity of cells exist within a tumor and that a single therapeutic approach may not likely be effective in killing all cancer cells. It is also a possibility that these seemingly conflicting theories may not be entirely mutually exclusive [13,19]. It seems possible, with the countless combination of mutations and epigenetic factors that may coordinate to result in neoplasia, that some cancers may develop by overriding natural stem cell differentiation hierarchies, whereas others may develop by self-reinforcing and runaway mutagenesis that allows for stochastic evolution and competition among the most malignant clonal populations. Others have proposed that perhaps the evolution of cancer itself may also allow for phases that alternate between hierarchical and stochastic patterns. For example, Tian et al. have suggested that as the epigenetic landscape of cancer cells becomes severely destabilized, hierarchical patterns that were previously evident in tumor progression may be overridden by aggressive clonal populations that have lost connection to that hierarchical architecture [20]. If cancer can ascribe to one of two driving mechanisms, or even worse, migrate between the two, then development of therapies to tackle the heterogeneity achievable within these scenarios will be even more crucial.

Other recent reports propose the concept of phenotypic equilibrium occurring in populations of cancer cells between stem and non-stem cancer cells. This hypothesis may also offer a reconciliation between the stochastic and hierarchical theories, or at least serve to further elucidate the confusing array of cancer cell behavioral data. Gupta et al. report data suggesting that breast cancer stem cells, luminal cells, and basal cells (defined by antigen expression profiles) can stochastically transition between these states following a Markov model [21]. This model asserts that cells expressing a certain phenotype will exhibit distinct probabilities of either remaining in that state or transitioning to another state. Not all transition probabilities are equal, but with time and a stable environment, this model predicts a steady equilibrium ratio of each phenotype existing within the population. Moreover, these findings suggest that while rare, it is possible for non-stem cells to convert to stem cells. Iliopoulos et al. also report a similar finding that non-stem cells can convert back to cancer stem cells in response to the secreted signal interleukin-6 [22]. These studies mostly used breast and some prostate cancer cells so it is not clear if this phenomenon will be observed in most cancers generally.

The equilibrium concept poses other challenges for heterogeneous drug targeting. In this case it would be important to target both stem and non-stem cells and perhaps more importantly develop therapeutics that can prevent the conversion of a non-stem cell to a stem cell, because the maintenance of a stem cell population seems to be of greatest concern when considering tumor recurrence. Gupta et al. also reported that drug sensitivity of the various phenotypes in a population may also follow a Markov model, and thus, this model may also be informative in developing new drug strategies against the heterogeneously shifting population [21].

3. Stromal cell contributions to tumor progression

Tumors consist of several non-transformed stromal cells, such as fibroblasts, endothelial cells, and immune cells, that are now understood to have important interactions with cancerous epithelial cells [4,5]. Fibroblasts associated with tumor cells, sometimes called carcinoma associated fibroblasts (CAFs), develop a phenotype distinct from normal fibroblasts, and can retain this phenotype for several passage doublings even when removed from the presence of carcinoma cells [23]. Unique aspects of this phenotype include an increase in the myofibroblastic marker alpha-smooth muscle actin (α-SMA) and the ability to contract collagen gels. These fibroblasts influence the growth of tumor cells by reciprocal paracrine signaling involving stromal cell-derived factor-1 (SDF-1) and transforming growth factor beta 1 (TGF-β1) [24]. Co-implantation xenograft studies of cancer cells with CAFs indicate that tumor volume will increase more quickly with the CAFs than would occur without them [23]. Tlsty and Cunha showed that tumorigenesis occurs when non-transformed epithelial cells were coinjected with CAFs, but not with normal fibroblasts [1]. This suggests that the CAFs have evolved an independent and stable phenotype that contributes to tumor progression.

The contribution of CAFs to tumor progression suggests that there may be some potential to target them for tumor therapy [25]. One potential obvious target would be to inhibit receptors or signaling molecules of the many soluble signals between fibroblasts and epithelial cells [4]. Further work is uncovering some specific cell markers associated with tumor stroma. One example is fibroblast activation protein (FAP), which is a protease within the dipeptidyl peptidase IV gene family. This protein has restricted expression in normal tissue, but is upregulated in some tissues during instances of tissue remodeling, wound healing, inflammation, as well as in fibroblasts of epithelial tumors and in sarcomas. The proteolytic capacity of this membrane protein could potentially contribute to matrix remodeling, which might play a role in angiogenesis and metastasis [26]. There is also some evidence that FAP expression may allow the tumor to evade anti-tumor immune response. A few studies have considered the use of monoclonal antibodies to FAP or the use of inhibitors to FAP protease activity as an approach to tumor therapy [27].

4. Heterogeneous conditions in the tumor microenvironment

The heterogeneity of the microenvironment across spatial regions of a tumor can also have a strong influence on the biology of individual tumor cells. Some of this is attributed to the proximity of a cell to gradient concentrations of paracrine factors released from other tumor cells or from stromal cells. It is also known that the extracellular matrix (ECM) can significantly influence cell behavior, and cancers are known to misregulate factors controlling the remodeling of their matrix or integrin receptors which are influenced by the ECM ligands [28]. The organization of the ECM is not likely to be consistent throughout a large, aggressive tumor [29]. Other variations in local environment are caused by accessibility to the content delivered by perfusion of plasma and blood cells within the circulatory system, including oxygen and endocrine signals [5].

One factor within the microenvironment that has received substantial investigation over recent years is the effect of hypoxia on tumor cells. As tumor growth is often rapid and unorganized, cells within the center of a solid tumor typically will experience some level of hypoxia because new blood vessel formation will not be well coordinated with new bulk formation, leaving interior cells severely isolated and lacking access to adequate oxygen and nutrients. Various investigations have revealed that hypoxia is an important but complex factor affecting cancer cells, and clinical testing shows that up to 50–60% of locally advanced solid tumors have heterogeneously distributed regions of significant hypoxia [30]. While it would seem that limited oxygen would be a fortuitous happenstance to prevent runaway proliferation of cancer cells, it is also known that hypoxia can influence the metastatic and stem-like properties of cells [31,32]. This may explain the unfortunate finding that hypoxic

tumors tend to correlate with a worse prognosis, at least in some cancers [30].

Hypoxia has been shown to correlate with resistance to both radiation and chemotherapy [33]. There are a number of reasons why hypoxia may facilitate drug resistance in a tumor. First, hypoxia is usually caused by poor vascularization and diffusion limitations, and any drug delivery through the blood stream will have difficulty reaching cells in hypoxic regions. Furthermore, cells are shown to reduce proliferation rates in low oxygen and thus these cells may evade therapies that target mitotic mechanisms. Also, it appears that along with increased quiescence in low oxygen, cells may also revert or maintain any stem cell-like properties, perhaps invoking the issues of cancer stem cells discussed above [32]. This may be a parallel pattern to adult stem cells that are activated to help heal ischemia-damaged tissue. Hypoxia appears to promote evolution of cancer cells with capacity to metastasize as well as withstand nutritive deprivation, both of which might also help facilitate mechanisms of drug resistance or evasion [31].

5. Conclusion

Here we have only briefly reviewed some of the many factors that can contribute to heterogeneity within a tumor. Whether cancer cells follow a hierarchical, stochastic, or equilibrium model, it is evident that significant diversity exists among the population of transformed cells. This diversity is further compounded by factors in the microenvironment such as paracrine signaling from associated stromal cells or from hypoxic conditions. It is not easily observed whether genetic, epigenetic, or microenvironmental influencers may have the most impact on the fate of a cancer cell. Each malignant cell may be influenced by a different ratio of these factors at a given time. Furthermore, the signals coming to any cell from each of these drivers is not steady and will fluctuate with time, creating a very dynamic and difficult process to correct or shutdown. Nevertheless, future therapies must correctly diagnose and deploy to these multiple needs.

As researchers in the treatment of cancer, we must move collectively to acknowledge the rampant, dynamically evolving heterogeneity found in most tumors. We must embrace this complexity and ensure that future studies continue to gather the necessary information to allow for improved therapeutic options. This will obviously include further investigation of cancer biology, but will likely require the development of new technology or techniques that enable us to test more relevant hypotheses in areas where only limited assays are available. These enabling technologies will require collaboration of scientists and engineers with different expertise to create new technologies or to facilitate accurate interpretation of data obtained in different disciplines. While tumor heterogeneity may appear discouraging for the development of successful treatment strategies, it may also reveal new approaches and tunable vulnerabilities that can be exploited to achieve success. It is likely that therapies may need to be catered to the unique dynamics of each patient and a multipronged approach to specifically address the most relevant issues for each disease. Recognizing greater challenges should not be a reason for reduced optimism. It should be a reason for great optimism as the ability to handle tumor heterogeneity is the essence of the personalized medicine we are striving to achieve.

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References

[1] T.D. Tlsty, L.M. Coussens, Tumor stroma and regulation of cancer development, Annu. Rev. Pathol. 1 (2006) 119–150.
[2] S.S. McAllister, A.M. Gifford, A.L. Greiner, S.P. Kelleher, M.P. Saelzler, T.A. Ince, F. Reinhardt, L.N. Harris, B.L. Hylander, E.A. Repasky, R.A. Weinberg, Systemic endocrine instigation of indolent tumor growth requires osteopontin, Cell 133 (2008) 994–1005.
[3] S.M. Cabarcas, L.A. Mathews, W.L. Farrar, The cancer stem cell niche—there goes the neighborhood? Int. J. Cancer 129 (2011) 2315–2327.
[4] N.A. Bhowmick, E.G. Neilson, H.L. Moses, Stromal fibroblasts in cancer initiation and progression, Nature 432 (2004) 332–337.
[5] K. Polyak, I. Haviv, I.G. Campbell, Co-evolution of tumor cells and their microenvironment, Trends Genet. 25 (2009) 30–38.
[6] A. Prat, C.M. Perou, Deconstructing the molecular portraits of breast cancer, Mol. Oncol. 5 (2011) 5–23.
[7] J.S. Reis-Filho, L. Pusztai, Gene expression profiling in breast cancer: classification, prognostication, and prediction, Lancet 378 (2011) 1812–1823.
[8] I. Vanden Bempt, M. Drijkoningen, C. De Wolf-Peeters, The complexity of genotypic alterations underlying HER2-positive breast cancer: an explanation for its clinical heterogeneity, Curr. Opin. Oncol. 19 (2007) 552–557.
[9] Y.P. Choi, H.S. Shim, M.Q. Gao, S. Kang, N.H. Cho, Molecular portraits of intratumoral heterogeneity in human ovarian cancer, Cancer Lett. 307 (2011) 62–71.
[10] J. Liu, S.K. Lau, V.A. Varma, R.A. Moffitt, M. Caldwell, T. Liu, A.N. Young, J.A. Petros, A.O. Osunkoya, T. Krogstad, B. Leyland-Jones, M.D. Wang, S. Nie, Molecular mapping of tumor heterogeneity on clinical tissue specimens with multiplexed quantum dots, ACS Nano 4 (2010) 2755–2765.
[11] L. Ding, M.J. Ellis, S. Li, D.E. Larson, K. Chen, J.W. Wallis, C.C. Harris, M.D. McLellan, R.S. Fulton, L.L. Fulton, R.M. Abbott, J. Hoog, D.J. Dooling, D.C. Koboldt, H. Schmidt, J. Kalicki, Q. Zhang, L. Chen, L. Lin, M.C. Wendl, J.F. McMichael, V.J. Magrini, L. Cook, S.D. McGrath, T.L. Vickery, E. Appelbaum, K. Deschryver, S. Davies, T. Guintoli, R. Crowder, Y. Tao, J.E. Snider, S.M. Smith, A.F. Dukes, G.E. Sanderson, C.S. Pohl, K.D. Delehaunty, C.C. Fronick, K.A. Pape, J.S. Reed, J.S. Robinson, J.S. Hodges, W. Schierding, N.D. Dees, D. Shen, D.P. Locke, M.E. Wiechert, J.M. Eldred, J.B. Peck, B.J. Oberkfell, J.T. Lolofie, F. Du, A.E. Hawkins, M.D. O'Laughlin, K.E. Bernard, M. Cunningham, G. Elliott, M.D. Mason, D.M. Thompson Jr., J.L. Ivanovich, P.J. Goodfellow, C.M. Perou, G.M. Weinstock, R. Aft, M. Watson, T.J. Ley, R.K. Wilson, E.R. Mardis, Genome remodelling in a basal-like breast cancer metastasis and xenograft, Nature 464 (2010) 999–1005.
[12] A.P. Albino, K.O. Lloyd, A.N. Houghton, H.F. Oettgen, L.J. Old, Heterogeneity in surface antigen and glycoprotein expression of cell lines derived from different melanoma metastases of the same patient. Implications for the study of tumor antigens, J. Exp. Med. 154 (1981) 1764–1778.
[13] M. Shackleton, E. Quintana, E.R. Fearon, S.J. Morrison, Heterogeneity in cancer: cancer stem cells versus clonal evolution, Cell 138 (2009) 822–829.
[14] J.E. Dick, Stem cell concepts renew cancer research, Blood 112 (2008) 4793–4807.
[15] A. Lorico, G. Rappa, Phenotypic heterogeneity of breast cancer stem cells, J. Oncol. 2011 (2011) 6 Article ID 135039.
[16] E. Charafe-Jauffret, C. Ginestier, F. Iovino, J. Wicinski, N. Cervera, P. Finetti, M.H. Hur, M.E. Diebel, F. Monville, J. Dutcher, M. Brown, P. Viens, L. Xerri, F. Bertucci, G. Stassi, G. Dontu, D. Birnbaum, M.S. Wicha, Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature, Cancer Res. 69 (2009) 1302–1313.
[17] M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance, Nat. Rev. Cancer 5 (2005) 275–284.
[18] C.E. Eyler, J.N. Rich, Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis, J. Clin. Oncol. 26 (2008) 2839–2845.
[19] M. Shipitsin, L.L. Campbell, P. Argani, S. Weremowicz, N. Bloushtain-Qimron, J. Yao, T. Nikolskaya, T. Serebryiskaya, R. Beroukhim, M. Hu, M.K. Halushka, S. Sukumar, L.M. Parker, K.S. Anderson, L.N. Harris, J.E. Garber, A.L. Richardson, S.J. Schnitt, Y. Nikolsky, R.S. Gelman, K. Polyak, Molecular definition of breast tumor heterogeneity, Cancer Cell 11 (2007) 259–273.
[20] T. Tian, S. Olson, J.M. Whitacre, A. Harding, The origins of cancer robustness and evolvability, Integr. Biol. 3 (2011) 17–30.
[21] P.B. Gupta, C.M. Fillmore, G. Jiang, S.D. Shapira, K. Tao, C. Kuperwasser, E.S. Lander, Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells, Cell 146 (2011) 633–644.
[22] D. Iliopoulos, H.A. Hirsch, G. Wang, K. Struhl, Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 1397–1402.
[23] A. Orimo, P.B. Gupta, D.C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V.J. Carey, A.L. Richardson, R.A. Weinberg, Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell 121 (2005) 335–348.
[24] Y. Kojima, A. Acar, E.N. Eaton, K.T. Mellody, C. Scheel, I. Ben-Porath, T.T. Onder, Z.C. Wang, A.L. Richardson, R.A. Weinberg, A. Orimo, Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20009–20014.
[25] P. Micke, A. Ostman, Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy? Lung Cancer 45 (Suppl. 2) (2004) S163–S175.
[26] T. Kelly, Fibroblast activation protein-alpha and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy, Drug Resist. Updat. 8 (2005) 51–58.
[27] D.M. Yu, T.W. Yao, S. Chowdhury, N.A. Nadvi, B. Osborne, W.B. Church, G.W. McCaughan, M.D. Gorrell, The dipeptidyl peptidase IV family in cancer and cell biology, FEBS J. 277 (2010) 1126–1144.

[28] C.C. Park, M.J. Bissell, M.H. Barcellos-Hoff, The influence of the microenvironment on the malignant phenotype, Mol. Med. Today 6 (2000) 324–329.
[29] A. Marusyk, K. Polyak, Tumor heterogeneity: causes and consequences, Biochim. Biophys. Acta 1805 (2010) 105–117.
[30] P. Vaupel, A. Mayer, Hypoxia in cancer: significance and impact on clinical outcome, Cancer Metastasis Rev. 26 (2007) 225–239.
[31] P. Vaupel, L. Harrison, Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response, Oncologist 9 (Suppl. 5) (2004) 4–9.
[32] Y. Kim, Q. Lin, P.M. Glazer, Z. Yun, Hypoxic tumor microenvironment and cancer cell differentiation, Curr. Mol. Med. 9 (2009) 425–434.
[33] P. Vaupel, A. Mayer, Hypoxia and anemia: effects on tumor biology and treatment resistance, Transfus. Clin. Biol. 12 (2005) 5–10.