

The Roles of Microenvironment in Tumor Dormancy

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Abstract: Several studies have shown that metastatic cancer cells may lie dormant in a secondary site for more than a decade, all the while retaining the ability to aggressively reemerge. Once these dormant cancer cells regrow, cancer becomes incurable, yet the mechanisms that instruct metastatic cancer cells to become dormant and later escape from dormancy still remain unknown. In this review we will discuss the known regulatory roles of the microenvironment in tumor dormancy and some potential models for future studies of tumor dormancy. Better understanding of the mechanisms involved has the potential to lead to significant improvements in the care of advanced cancer patients and the prevention of cancer recurrence.

Keywords: Microenvironment; Tumor dormancy; Xenograft model; Fluorescent dye

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Introduction

Certain mammals hibernate to endure the harsh conditions of winter. Hibernation is possible because energy is conserved when the metabolism is suppressed in response to stress. This is achieved when the basal body temperature, breathing and heart rate of an organism are allowed to drop. A similar process occurs at the cellular level with cancer. Cancer often recurs after long disease-free intervals, during which some cancer cells may lie dormant (or “hibernating”), and thus resistant to treatments that mainly target actively dividing cells [1]. Clinically, metastasis remains a major cause of death in cancer patients. Several studies have demonstrated that cells shed from a primary tumor, referred to as Disseminated Tumor Cells (DTCs), can remain dormant in distant tissues for long periods of time before eventually developing into full-blown metastases [2-4]. Understanding the mechanisms of tumor dormancy in distant tissues is therefore essential for the development of effective means of preventing and treating metastatic cancer.

Seeds require certain soils for robust growth [5]. Recent studies revealed that prostate cancer cells

target the specific site in the marrow where Hematopoietic Stem Cells (HSCs) reside, and subsequently displace HSCs to establish metastatic foci [6]. HSCs are believed to reside in a quiescent, or dormant state within this microenvironment, which is called the “niche,” until they are recruited for differentiation [7]. In fact, growing evidence suggests that prostate cancer cells parasitize the niche once they reach the marrow [8]. Since prostate cancer cells use similar mechanisms as HSCs in order to gain access to the marrow microenvironment [9-13], DTCs may also utilize the mechanisms that keep HSCs in a quiescent state to become dormant within the niche.

Although these studies hint at the possible mechanisms of tumor dormancy within the microenvironment, the processes by which actively growing cancer cells become dormant and later how dormant cancer cells reawaken into aggressive re growth are still largely unknown. This is in part due to the limitations of current technologies. In this review we will discuss the known regulatory roles of the microenvironment in tumor dormancy and some potential models for studying this process.

Microenvironment and Tumor Dormancy

It is believed that the niche surrounding stem cells regulates their dormancy by itself maintaining a dormant state [14]. The same is especially true in hematopoiesis [15,16]. Stem cells remain dormant within the niche as long as the niche is dormant, however, once the niche differentiates, stem cells also leave dormancy [17]. These observations suggest that the microenvironment plays a crucial role in maintaining stem cell dormancy. It has been shown that cancer cells use a process equivalent to that of HSC homing to the bone marrow during

dissemination [10,18], so it is reasonable to propose that DTCs may also utilize similar mechanisms to HSCs to become dormant. In addition, we recently revealed that DTCs from primary prostate cancer directly target and co-opt the HSC niche during dissemination, where they survive indefinitely by reversibly entering a dormant state [6]. Yet, how the microenvironment regulates tumor dormancy remains unclear.

It has been shown that Growth Arrest-Specific 6 (GAS6) expressed by the bone marrow microenvironment is an important molecule in maintaining HSC dormancy [19]. Likewise, GAS6 within the bone marrow helps to control prostate cancer cell dormancy [20]. GAS6 slows prostate cancer cell proliferation without inducing apoptosis, resulting in enhanced resistance to cytotoxic treatments [20]. Interestingly, once in the bone marrow metastatic prostate cancer cells increase GAS6 receptor Axl expression [20]. Recent findings revealed that the expression of GAS6 receptor Tyro3 is enhanced in rapidly dividing prostate cancer cells, whereas slowly dividing prostate cancer cells express relatively high levels of Axl [21]. These findings suggest that a balance between Axl and Tyro3 expression may serve as a molecular switch between prostate cancer dormancy and recurrence of bone metastases [21]. Similarly, the bone marrow stromal cell secreted Bone Morphogenetic Protein 7 (BMP7) reversibly inhibits the growth of prostate cancer stem-like cells through BMP receptor 2 (BMPR2) [22]. Here, the dormant state is induced by the activation of p38 mitogen-activated protein kinase, the cell cycle inhibitor p21, and the metastasis suppressor gene N-myc Downstream-Regulated Gene 1 (NDRG1) [22]. In addition, it was demonstrated that the ratio of Extracellular Signal-Regulated Kinase (ERK) and p38 pathways is also

important in the determination of tumor dormancy [23,24]. These results suggest that the BMP7-BMP2-p38-NDRG1 axis is another potential mechanism whereby prostate cancer cells become dormant in the bone marrow [22].

Cancer cells become dormant not only when they reach metastatic sites, but also during the actual process of metastasis. In a breast cancer model, DTCs reside in the microvasculature during dissemination [25]. The microvasculature becomes dormant (stable microvasculature) when Thrombospondin-1 (TSP-1) is activated, and both Transforming Growth Factor Beta-1 (TGF- β 1) and periostin are down regulated [25]. Similar to normal stem cells [17], breast cancer cells are dormant when in contact with stable microvasculature [25]. In contrast, dormant cancer cells revive when microvasculature becomes active (sprouting neovasculature) [25], yet another example of the control of microenvironment on dormancy and metastasis.

Micro environmental regulation of dormancy is also observed in primary sites. Prostate cancer stem-like cells can be found in the proximal region of prostatic ducts. This region expresses high levels of TGF- β 1, which causes both the proximal ductal region and prostate cancer stem-like cells to stay dormant by over expressing the *apoptosis suppressor gene bcl-2* [26]. Despite contrasting effects, TGF- β 1 is clearly a critical molecule employed by the microenvironment in the regulation of tumor dormancy, and its effects may be specific to the particular microenvironment.

Methods for Monitoring Tumor Dormancy within the Microenvironment

To understand the biology of tumor dormancy

within the microenvironment, better technologies must be developed to track cellular proliferation in real time. Cell cycle analyses are widely used to characterize cell proliferation, however these assays cannot elucidate whether a cell is truly dormant, because they merely supply a single snapshot within a larger, complicated process. The thymidine analog Bromodeoxyuridine (BrdU) is also used to analyze cell proliferation. BrdU synthesizes a traceable nucleoside which distributes equally between daughter cells during cell division. This technique is well suited to monitor the kinetics of cellular proliferation, but requires cellular fixation and/or membrane permeabilization prior to analysis. These processes greatly compromise the ability to further study any recovered cells, therefore a practical method is needed to isolate these cells without perturbing biological functions.

Fluorescent dye is known to be a useful tool to trace cell proliferation. Like BrdU, it is distributed equally between daughter cells, however the stained cells are often viable upon recovery as no fixation or permeabilization is necessary. There are three types of fluorescent dyes; DNA-binding, cytoplasmic, and membrane-bound. DNA-binding dyes [27] and cytoplasmic dyes [28-30] have been used to analyze cell migration and proliferation. Unfortunately, these dyes have been found to have direct inhibitory effects on normal cell division [31-35]. Although further studies are clearly warranted, the effects of DNA-binding and cytoplasmic dyes on cell migration and proliferation need to be considered whenever these dyes are used. Alternatively, membrane-bound dyes can be used in cell migration and proliferation assays due to their minimal toxicity [36, 37] and long-term retention [38-40]. Consistent with these findings, our recent studies have demonstrated that a membrane-bound fluorescent dye, DiD, is an

adequate tool for tracking cell proliferation and migration without interfering with cellular functions. In this study, less DiD retention correlated with higher proliferative and mitotic activity than cells which retained higher levels of DiD. Critically, DiD had no *adverse* effects on cell proliferation, migration, or apoptosis. More importantly, the retention of DiD was observed after 3 weeks of *in vivo* subcutaneous tumor implantation. With that in mind, membrane-bound fluorescent dyes are a very promising area for further research, however there is a potential pitfall. Unavoidably, dyes can be transferred from stained cells to surrounding cells during apoptosis. Therefore, methods that recognize cell viability need to be used in combination with these dyes in order to be truly useful.

To study the interaction between cancer cells and the surrounding microenvironment, we need to be able to distinguish between these two cell types. When human cancer cells are engrafted in murine tissues (xeno graft model), we are able to do this with relative ease. Using an antibody specific to Human Leukocyte Antigen (HLA), human metastatic tumor cells can be isolated from murine tissues by fluorescence-activated cell sorting (FACS) in several xenograft models [6,41]. The combination of i) monitoring cell proliferation with membrane-bound fluorescent dyes and ii) isolating viable human cells from murine microenvironment tissues may be useful in alleviating current. These concepts address the daunting problem of latent metastases from a much needed new direction. We must identify the mechanisms by which DTCs become and stay dormant for extended periods of time before resurfacing with

technical difficulties and further our understanding of the biology of tumor dormancy within the microenvironment. However, these techniques are limited in scope, and further investigations will be needed to apply what is learned from *in vitro* and animal model experiments to clinically based studies.

Conclusions

Although the early diagnosis and treatment of primary tumors has increased the prevalence of long disease-free periods experienced by cancer patients, certain cancers still metastasize and recur, often with fatal results. However, our explorations into the mechanisms of tumor dormancy are in their early stages. We must discover more about the enemy before we can expect to win the war against cancer.

In this review, we discussed the known regulatory roles of the microenvironment in tumor dormancy (Figure 1). We also outlined several techniques that may help to advance progress in this challenging field. Until better technologies, which translate well into clinical study are available, we are limited to xenograft models designed for the study of human cancer cell dormancy. Additionally, using the current models, cell membrane binding fluorescent dye scan be used to monitor cell proliferation, which may have profound and positive impacts on defining the molecules that are responsible for establishing both tumor dormancy and recurrence.

catastrophic effects. This will not only lead to a greater understanding of microenvironment supported tumor dormancy and recurrence, but will also provide gateways to new treatment strategies.

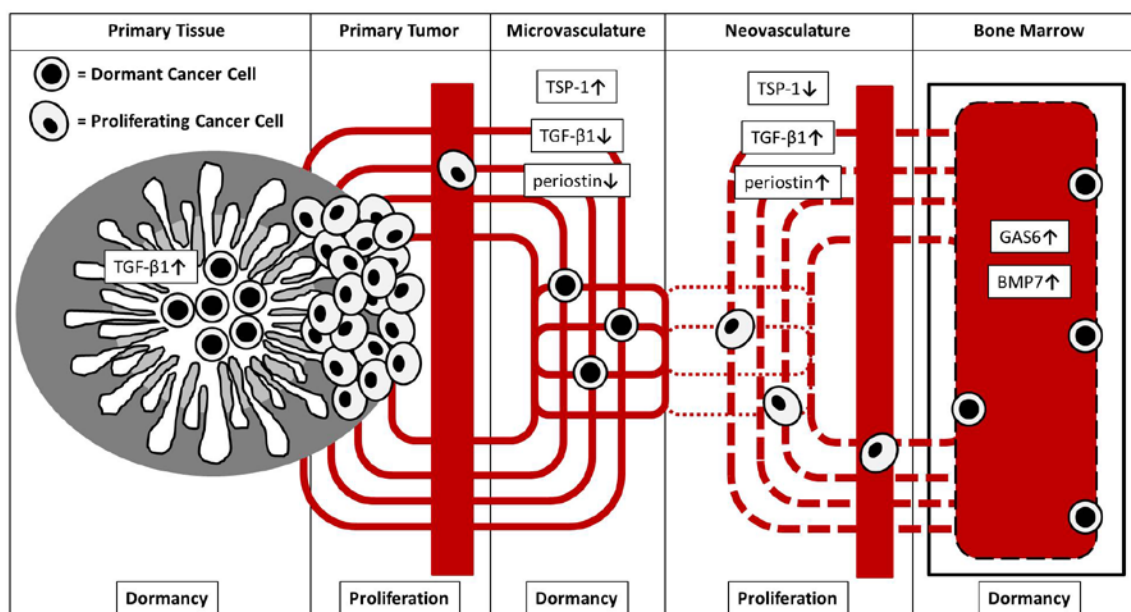


Figure 1: Mechanisms behind microenvironment derived cancer cell dormancy regulation.

Dormant cancer cells can be found in all stages of cancer, from primary tumors to secondary metastases. In the primary tissue, high levels of TGF-β1 are believed to cause enhanced expression of anti-apoptotic gene bcl-2, which in turn induces dormancy in resident cancer cells. In the microvasculature however, low levels of TGF-β1 and perostin caused by expression of TSP-1 keep the surrounding tissue stable and disseminated

tumor cells (DTCs) dormant. When the microvasculature activates and sprouts neovasculature, resident cancer cells also switch from a dormant to a proliferative state. Once in the bone marrow, a major metastatic destination, high levels of GAS6 in the hematopoietic stem cell (HSC) niche and high levels BMP7 in the stroma cause dormancy in DTCs by arresting growth and inhibiting apoptosis.

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