REVIEW

Cancer initiation and progression within the cancer microenvironment

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Abstract

Within the cancer microenvironment, the growth and proliferation of cancer cells in the primary site as well as in the metastatic site represent a global biological phenomenon. To understand the growth, proliferation and progression of cancer either by local expansion and/or metastasis, it is important to understand the cancer microenvironment and host response to cancer growth. Melanoma is an excellent model to study the interaction of cancer initiation and growth in relationship to its microenvironment. Social evolution with cooperative cellular groups within an organism is what gives rise to multicellularity in the first place. Cancer cells evolve to exploit their cellular environment. The foundations of multicellular cooperation break down in cancer because those cells that misbehave have an evolutionary advantage over their normally behaving neighbors. It is important to classify evolutionary and ecological aspects of cancer growth, thus, data for cancer growth and outcomes need to be collected to define these parameters so that accurate predictions of how cancer cells may proliferate and metastasize can be developed.

Keywords Cancer microenvironment · Cancer evolution · Natural selection · Cancer ecology

Introduction

Stanley P. Leong

The major thesis in the Origin of Species [1] according to Darwin, published in 1859, consists of mutations within the living organisms, natural selection from the environment upon the mutated organisms resulting in the fittest individuals to survive in the new environment. About 94 years later, the double helix model of DNA of Watson and Crick [2] established the genetic and molecular mechanisms for mutations as hypothesized by Darwin. In the past several decades, with enormous achievements in cancer research with completion of the human genome project identifying 30,000 genes in 23 pairs of chromosomes (http://www.humangenomeproject), the hallmarks of cancer development have been well summarized by Hanahan and Weinberg [3] namely (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) avoiding immune destruction, (4) deregulating cellular energetics, (5) enabling replicative immortality, (6) inducing angiogenesis, (7) resisting cell death and (8) activating invasion and metastasis. These newly acquired cellular activities resulting from DNA changes including deletions, amplifications, mutations, translocations and epigenetic alterations provide a survival and/or a reproductive advantage over their normal counterparts, allowing them to expand within the normal tissue and replace neighboring cells. Despite the potential resistance of the host microenvironment to cancer growth, over time, the cancer cells outsmart the microenvironment as they adapt to their microenvironmental milieu.

Cancer is heterogeneous both genetically and phenotypically. It is important to understand the interaction between heterogeneous cancer cells within the cancer population as well as their interaction with their microenvironment (https://mbi.osu.edu/event/?id=819), which includes the non-cancerous stromal cells present within or adjacent to the cancer cells. The cancer microenvironment consist of several types of cells that modulate cancer cells during their progression.

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Hanahan and Coussens [4] has listed four major stromal cell types within the cancer microenvironment namely: (1) infiltrating immune cells, (2) cancer-associated fibroblastic cells, (3) endothelial cells and (4) pericytes. Their multi-faceted functions may favor the growth of cancer cells. In addition, the metabolites or proteins produced by these cells may influence the growth of the cancer cells. The cancer microenvironment [5] may serve as the ecology in which cancer cells are selected for survival and proliferation. Thus, the Darwinian evolutionary concept being developed in the Origin of Species [1] can be generalized and applied to cancer evolution [6–8].

One of the major systems to keep the cancer cells at bay is the host immune system, though, when it becomes dysfunctional, immune protection may be rendered ineffective allowing cancer to grow. These dysfunctions may include decreased antigen presentation, altered immune cell trafficking, chronic inflammation, chronic T cell receptor signaling defects, immunosuppression, metabolic competition to suppress immune activities and cancer mutations to escape immune response [9]. Within the cancer microenvironment, the infiltrated fibroblasts lay down extracellular matrix proteins, which give rise to the fibrotic characteristics of the cancer. Further, growth factors and proteases like matrix metalloproteinases are produced for wound healing, angiogenesis and tissue remodeling. Other matrix proteins such as laminin, tenascin and fibronectin are also produced, which may enhance cancer growth. The fibroblasts within the cancer microenvironment, so called cancer-associated fibroblasts, promote growth, angiogenesis inflammation and metastasis in cancer cells [10, 11]. Within the extracellular matrix in the tissue microenvironment, a predominant glycosaminoglycan known as hyaluronic acid is important for homeostasis and signaling functions for normal cellular functions. However, within the cancer microenvironment, hyaluronic acid may cause tumorigenesis and metastasis [12, 13]. It may increase tumor interstitial pressure, which may cause compression of vasculature resulting in compromised vascular flow (hypoxia) [14] and lymphatic function (immunosuppression) [15, 16]. In addition, hyaluronic acid has been found to bind with CD44 [17] with resultant activation of downstream signaling pathways MAPK, Rac and P13K, which are important for cancer cell survival and proliferation.

Thus, the Darwinian concept of biological evolution may be applied, in general, to the development, progression and evolution of cancer. We will divide this review article into three sections. Using melanoma as a model, Stanley Leong attempts to make the case that melanoma undergoes an evolutionary process to become more aggressive. Athena Aktipis will addresses the social evolution and natural selection in cancer progression. Carlo Maley describes and classifies the evolution and ecology of tumors.

Interactions between cancer and stromal cells: selection of the fittest clones to metastasize

Stanley P. Leong

Using melanoma as a model, the cancer evolution may be demonstrated to follow the general principles of Darwinian evolutionary concept. Melanoma is a cancer derived from the transformation of melanocytes [18–20] in the skin primarily and occasionally in the mucosa. Melanoma is a heterogeneous disease from different body locations such as cutaneous, uveal, acral and mucosal sites with different clinical outcomes and molecular abnormalities. Various mutational profiles and unique risk factors have been found to be associated with these different characteristics [21].

Melanoma occurs predominantly in the lightly pigmented racial populations such as the Caucasians with the incidence of melanoma in the United States in 1 out of 74 in 2000 with 70,000 new cases and 8,000 deaths per year [22]. Melanin, a pigment product of the melanocyte, is actually a sun blocker. This important molecule is the basis of evolution of human pigmentation (http://www.youtube.com/watch?v=d4KcRMTKImQ). In general, melanoma undergoes several stages of progression from the benign nevus to dysplastic nevus to melanoma with radial and vertical growth phase being associated with proliferation and invasion. Molecular studies of melanoma precursor lesions of melanoma indicate that melanoma progresses from benign nevi to dysplastic nevi, to melanoma in situ and eventually to invasive and metastatic melanoma [21].

Melanoma cells have been shown to secrete several autocrine and paracrine growth factors which are associated with tumor neovascularization and metastasis. Further, these factors may induce endothelial cell proliferation, capillary tube formation and angiogenesis via interactions between vascular endothelial growth factors (VEGF-A and B) and their receptors, VEGFR-1 and 2 [23]. Hirakawa et al. have found de novo lymphangiogenesis by VEGF-C in sentinel lymph nodes (SLNs) to promote tumor metastasis [24]. Using a transgenic mice with overexpression of VEGF-C and green fluorescent protein specifically in the skin, the effects of chemically-induced skin carcinogenesis in this model were evaluated. They found that VEGF-C induced proliferation of lymphatic networks within SLNs, even prior to metastasis. Once the metastatic cells reached the SLNs, lymphangiogenesis in these lymph nodes had increased. Of significance, in mice with metastatic SLNs, tumor expressing VEGF-C were more likely to metastasize to additional organs, such as distal lymph nodes and lungs. There were no metastases

in distant organs when the lymph node showed no metastasis. Thus, these findings have demonstrated the significant role of VEGF-C role in the lymphangiogenesis within the SLNs as well as facilitation of systemic cancer metastasis. Further, Karaman and Detmar have provided evidence that lymphatic vessels may actively recruit cancer cells to lymph nodes [25]. Two recent mouse studies have shown that tumor cells in the SLN may spread directly into the systemic vasculature [26, 27]. In these experimental mouse metastasis models, tumor cells were shown, by extrapolation, to invade blood vessels within the SLN, enter the blood circulation and spread to the lungs.

Figure 1 shows the relationship of a growing melanoma in its cancer microenvironment. In the pre-SLN era, regional lymph node metastasis has been described as an indicator rather than governor in the progression of cancer [28]. This concept may be challenged in the SLN era as most melanoma [29] and breast cancer [30] patients with micrometastasis in their SLNs may enjoy a good survivorship after the resection of their SLNs with metastatic cancer cells. Perhaps, it is a spectrum of events from the initial arrival of cancer cells in the SLNs, which function like an incubator [31, 32]. Thus, in general, the SLNs may serve as a gateway prior to regional lymph node or systemic metastasis. In
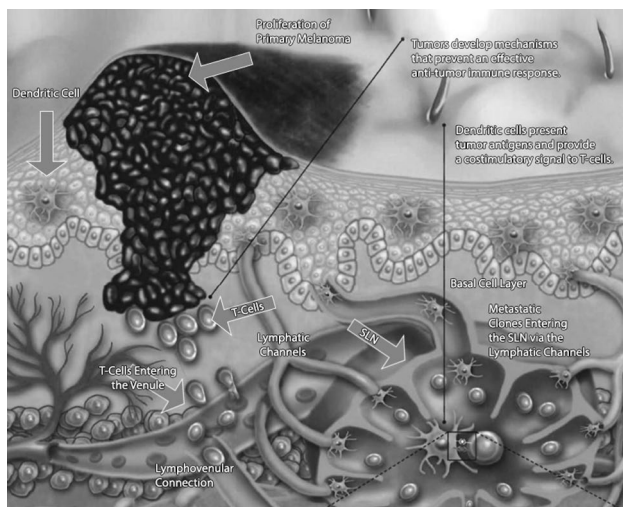
about 10% of melanoma patients, cancer cells may spread to the distant sites bypassing the SLNs. These patients have negative SLNs and yet develop systemic metastasis at a later time. Overall, the patterns of metastasis for melanoma may be summarized in Fig. 2 showing that SLN may be the primary gateway for primary melanoma to spread.

Melanoma is biologically and molecularly heterogeneous, as a result of intrinsic mutations, mostly from the UV excitations on the melanocytes. Within the melanoma microenvironment, selective forces, such as fibroblasts and other stromal cells as well as hyaluronic acid may act as Darwin's natural selection, modulating and selecting out the "fittest" clones to invade and metastasize. Dynamic changing relationship between the immune cellular profiles and different melanoma clones may result in compromised immune reactivity with melanoma clones escaping the immune surveillance resulting in uncontrolled growth and proliferation of the aggressive clones as in the PD-1 and PD-L1 interaction [33]. It requires the check point inhibitor such as an anti-PD-1 or anti-PD-L1 to break and overcome this immune blockade. Thus, early diagnosis and elimination of the primary melanoma are essential to a successful therapeutic strategy.

Recent explosive findings in T cell responses to cancer have discovered multiple co-stimulatory and inhibitory interactions [33]. In particular, the findings of check point inhibitors such as CTL-4 and PD-1 molecules on the T cells within the melanoma microenvironment. The use of monoclonal antibodies against such T cell molecules has

Fig. 1 Melanoma microenvironment. Using melanoma as a model, this figure (modified from an unidentified source of art work depicting the cancer microenvironment of a primary melanoma) shows the growing melanoma with invasion through the basement into the papillary and reticular junction of the dermis with a rich network of vascular and lymphatic channels. As the cancer cells proliferate, they develop mechanisms to block an effective anti-tumor activity by immune cells consisting of dentritic and T cells. The invading cells usually would enter the lymphatic channels and spread to the SLN. In the minority of the cases, they may invade the vascular channels either directly or through the lymphovenular channels and spread to systemic sites. The challenge is to determine the molecular mechanisms of how cancer clones spread either through the lymphatic or the vascular channels

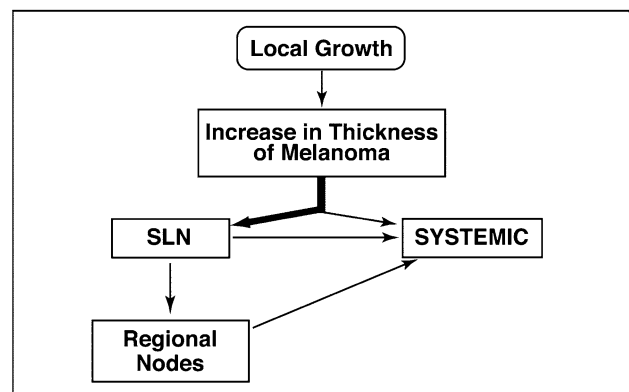## Paradigm of Metastasis for Melanoma

Fig. 2 Pathways of metastasis for melanoma. This figure shows the pathways of melanoma metastasis. Local growth and proliferation result in more aggressive clones, most of the time, the cancer cells would go through the lymphatic system into the SLN. The SLN may act as an incubator to allow the newly arrived cancer cells to grow and proliferate. Through the gateway of the SLN, the cancer cells may spread to the non-SLNs in the regional lymph node basin or to the systemic sites. In the minority of the cases, cancer cells from the primary site may spread to the systemic sites via the vascular channels

led to the revolutionary treatment results of ipilimumab and nivolumab and others against metastatic melanoma and perhaps, other types of cancer. A detailed summary of these recent milestones in the treatment of metastatic melanoma will be summarized by Swe and Kim [34].

With recent developments in molecular studies in lymphology and oncology, the intimate relationship between the immune system and cancer cells is further established and strengthened, perhaps, a new field that addresses this relationship is evident, and, here forth, I would like to coin it as oncolymphology, a field dedicated to the study of the cancer cells relating to proliferation, growth, metastasis and even dormancy in relationship to the structure and physiology of the lymphatic system. The interaction between the two fields in different types of cancer and different individuals may be intriguing. Such an interaction may be different in different patients because of heterogeneous genetic background both from the perspectives of the cancer cell and the immune system. With such a heterogeneous background, the interaction is further complicated by continuous dynamic changes rather than being static in that the cancer cell population may change over the course of the disease as well as the immune system. Unless we take these dynamic changes of biological heterogeneity both from the genetics of the cancer and immune cells, we will not be able to understand the complexity of cancer growth and cancer escape from the immune system. The cancer cells may evolve into such an aggressive form and the immune system may become tolerant and disabled to fight the newly evolved cancer clones resulting in the winning of the war by the cancer cells. To control the cancer growth and finally eliminate the cancer cells, it is critical for us to understand the dynamic interactive changes of the two systems on a molecular basis so that therapeutic maneuvers can be developed to deal with different patients relating to these dynamic interactive changes between their cancer cells and the immune system. Perhaps, with the full understanding of each patient's cancer in relationship to his or her immune system so that precision therapeutic maneuver may be delivered to overcome the cancer growth and finally eliminate it. The underlying principle of cancer dominance is akin to Darwin's survival of the fittest that develops under the influence of natural selection, in this case the cancer microenvironment acts as a form of natural selection to allow the development of the fittest cancer clone or clones. The challenge is to find out what are the conditions in the cancer microenvironment that may allow the emergence of the "fittest" or most aggressive cancer clones. Then, therapy may be developed to block these conditions either against the cancer cells directly or the stromal cells indirectly.

Social evolution and natural selection in cancer progression

Athena Aktipis

Cancer cells evolve through natural selection inside the environment of the body. Unfortunately, this process often results in the survival and proliferation of cells that exploit the body—cells that can initiate cancer and spur on its progression. Multicellular bodies, like ours, have evolved extremely high levels of cellular cooperation and coordination that enable us to perform complex behaviors. This cellular cooperation provided a strong evolutionary advantage for multicellular organisms that utilize it to outcompete other organisms [35]. Thus, social evolution among cells, favoring more cooperative cellular groups, is what gave rise to multicellularity in the first place.

However, large multicellular organisms are also susceptible to cellular cheating from within. In fact, the hallmarks of cancer [3] map onto cheating in the foundations of multicellularity that enabled multicellularity to evolve in the first place [36]. Multicellularity evolved because cells that cooperated had an advantage over those that didn't. The most evolutionarily successful cellular groups had within them cells that were capable of suppressing proliferation, controlling cell death, restraining resource use, dividing labor and creating and maintaining a health extracellular environment. Each of these foundations of multicellular cooperation break down in cancer.

Unfortunately, social evolution does not always lead to the optimal result for the (cellular) group. In the case of cancer evolution, cancer cells evolve to exploit their cellular environment. The foundations of multicellular cooperation break down in cancer because those cells that cheat have an evolutionary advantage over their normally behaving neighbors. Those cells that proliferate more quickly, avoid cell death, consume more resources, and cheat in the other foundations of multicellularity are more likely to survive and replicate, and the mutated genes coding for these cellular behaviors can subsequently expand in the cellular population. This leads to a cellular version of a classic social dilemma: what is best for the evolutionary fitness of the organism (the cellular group) is for cells to cooperate and restrain their behavior, but what is best for the evolutionary fitness of the cell is to exploit the cells around them and cheat in the foundations of multicellular cooperation. In other words, the body is like a giant tragedy of the commons: the body is a commons in which all the cells of the multicellular body live and function, and the phenomenon of cancer cells exploiting this multicellular commons is, quite literally, a tragedy.

When cancer cells overconsume resources in their environments, not only do they deprive nearby cells of those resources, they can also create conditions that favor cell motility and metastasis. In models that my colleagues and I have created, we found that cells that consumed resources at a faster rate evolved higher cell motility [37]. When cells use resources rapidly, this depletes the environment, making it harder for cells to survive without moving away from the depleted environment. This is parallel to the process of dispersal evolution that happens in ecology, where organisms that deplete the resources in their environments evolve to move more and disperse longer distances, than organisms that do not deplete their environments. In the case of cancer, this process of dispersal evolution may be contributing to the capacity of cells to move, not just in the primary tumor where they initially evolve, but also may be pre-adapting these cells to be better able to disperse for long distances within the body, leading to invasion and metastasis.

Social evolution inside neoplasms is not limited to the evolution of cellular cheating in the foundations of multicellularity, social evolution may also lead to selection for cooperation among cancer cells that may contribute to cancer progression. For example, groups of cells that can coordinate their signaling to better attract blood vessels or avoid immune predation will have an evolutionary advantage over those groups of cells that do not. Thus, social evolution may take place among micrometastases, where those micrometastases that are most effective at surviving and creating new micrometastases have the greatest evolutionary fitness [38]. Selection may also favor larger circulating cell clusters that can effectively stay together: experiments have found that cell cluster have a 23–50 fold advantage over single cells in creating metastases, and that these cell clusters are made of multiple clones [39]. Together, these findings suggest that social evolutionary processes may be critical during tumor dissemination and the development of metastases.

Classifying the evolution and ecology of cancers

Carlo Maley

One of the central problems we have in the management of both pre-cancers and cancers, is predicting how they will evolve. In the context of pre-cancers, this is called risk stratification. We would like to predict which pre-cancers are more likely to progress to invasion and metastasis, so that we can focus our interventions, with their concomitant costs and toxicities, on those people most likely to benefit. Equally important, we would like to reassure people with low risk pre-cancers and avoid the costs and toxicities of interventions for them. In the context of full blown cancers,

predicting how they will evolve is what we call prognosis, and furthermore, we would like to predict how they will evolve in response to an intervention, so that we can choose an optimal course of action.

Predicting evolution is difficult. We have learned for large scale sequencing efforts that even for a particular type of cancer, such as cutaneous melanoma, there are many different combinations of mutations that can cause a cancer. This makes it difficult to develop biomarkers for risk stratification or prognosis based on particular mutations. This problem derives from three facts: (1) evolution is stochastic. There is a great deal of randomness in which mutations occur in a cell; (2) there are many different mutations or other genetic and epigenetic alterations that can produce the same phenotype; (3) natural selection operates on phenotypes. So selection for the phenotypes of reproduction and survival in a tissue microenvironment results in different genetic and epigenetic alterations in different tumors, but similar phenotypes.

What we would like to know is the likelihood that a precancer will evolve malignancy and that a cancer will evolve to be lethal. A recent consensus statement from the community of evolutionary biologists and ecologists of cancer proposed that we should be able to classify tumors based on their likelihood of evolving malignancy or lethality [40]. The evolutionary trajectory of a tumor depends on both its evolvability and the selective pressures on that cell population. Selective pressures are determined by the ecology of the neoplastic cells. In broad strokes, the evolvability of a neoplasm is determined by its degree of genetic and epigenetic diversity within the neoplasm, and how that diversity changes over time. Diversity is the fuel for natural selection. The more variants there are in a population, the more opportunities for one to be more fit to the environment. However, diversity is not the whole story. A diverse population can be generated by a low mutation rate over a long period of time, or a high mutation rate over short period of time.

Ecology can be broadly split into resources and hazards. We predicted that profiling the resources and hazards of the neoplastic cells should also predict clinical outcomes [40]. There is already strong evidence that the immune cells in the microenvironment of a tumor provides predictive power for patient survival [41–50]. We have fewer assays and studies of the effects of resource abundance, turnover, and patchiness, on clinical outcomes. That will be an important priority for future studies.

We do not yet have enough data to determine which measures of diversity, change over time, hazards and resources are the most powerful predictors of clinical outcomes. The priority now is to collect that data. In order to do this, the ideal studies would involve multi-region sampling from tumors over time, with clinical outcomes. One could then test different measures of diversity, and each of the other

factors to determine which measure is most predictive and provides the most independent information when combined with measures of the other evolutionary and ecological factors. This should be developed in a training cohort and then validated in an independent cohort, to avoid over-fitting the statistical models on a single cohort.

Once we have an evolutionary and ecological classification system, we can design clinical trials to test for the best ways to manage the different classes of neoplasms, and also determine how different interventions change the evolutionary or ecological class of a neoplasm. Until now, we have lacked a language with which to describe the evolution of cancers. We need to develop such a language in order to make progress on both understanding and managing this evolutionary disease.

Conclusion

The molecular structure of the DNA double helix gives rise to potential mutations of the biological genetic materials for change and evolution. The major thesis of the Origin of Species in 1859 by Darwin [1] consists of mutation, diversity and natural selection being developed from his keen observations based on his careful collection and critical analysis of many specimens from Nature over time. Thus, the biological evolution by natural selection forms the major principle of diversity of living organisms. To understand the growth, proliferation and progression of cancer either by local expansion and/or metastasis, it is critical to understand the cancer microenvironment and host response to cancer growth. Thus, the cancer microenvironment may serve as a "natural selection" for the development of the "fittest cancer clone" to expand and metastasize.

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