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Studying cancer evolution and therapeutic responses in different organs: the pros and cons of a broad focus.

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Abstract

Cellular adaptation brought upon by insults such as old age and therapeutic exposure is a complex phenomenon in which cells undergo adaptive phenotypic changes. Our lab has focused on understanding the mechanisms underlying adaptation during the evolution of cancer, from the early stages of development to the ability of cancer cells to escape therapeutic challenges. Our studies span hematopoietic and lung systems. Herein, we discuss the advantages and disadvantages involved in studying two vastly different organ systems. Through the use of these organ/cancer model systems, we hope to develop interventions to limit oncogenic adaptation leading to cancer development and prevent adaption of cancers following treatment leading to cancer relapse.

Introduction

Somatic cells can adapt in changing conditions, which may be caused by contexts like old age or a history of cigarette smoking, as well as exposure to therapies. Similar to how bacterial strains adapt to antibiotics, contexts that lower cellular fitness will promote selection for cells that are genetically or epigenetically better adapted in the altered context. Exposures can have direct impacts on the fitness of both non-malignant and malignant cells, and (like aging) can also change selective pressures by altering tissue and tumor microenvironments. “Adaptation” can also describe the immediate activation of protective pathways and programs in response to an environmental change that improve the maintenance of cell survival or function. The mission of our lab is to study adaptive dynamics of somatic cells, from the initial evolution of malignancies to the ability of cancer

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cells to evade standard therapies, with the underlying theme of understanding how cells adapt (via selection for new phenotypes or through direct phenotypic change) under different contexts. To this end, our lab has developed mouse models to study the early stages of cancer development and leverages somatic cell genetics with human cancer samples to discover cancer vulnerabilities. Our lab initially focused on context-dependent leukemogenesis and leukemia therapeutics, but in the last decade has broadened our focus to include the study of the evolution of lung cancers and responses of these cancers to therapies. So how does a small lab span such different tissue systems and such different areas of cancer biology, from initiation to treatment?

Main Text

Old age is the dominant risk factor for cancers, with 90% of cancers diagnosed after the age of 50 (1). This pattern is clear for leukemias, although a small peak of incidence is evident for young children. For lung cancers, incidence rises sharply after mid-life, and interestingly, the shape of this curve is very similar for smokers and never-smokers – smoking greatly increases the risk of getting lung cancer, but does not appear to substantially alter *when* these cancers arise (2). Notably, while ~80% of lung cancers are caused by smoking, lung cancer in never-smokers still ranks as a top five cancer. Despite a clear connection between aging and cancer, old age is often overlooked as a principal risk factor in cancers (particularly for lung cancers). Furthermore, while mouse models have emerged as powerful tools to study cancer, few studies have utilized old mice to study either tumorigenesis or therapeutic development.

Elegant models have been developed to study both blood cancers and lung cancers initiated *de novo* in immune-competent mice. These models exhibit high penetrance and clinical behavior that mirror the human diseases. Additionally, xenograft and transgenic mouse models have been widely used to investigate tumorigenesis and its pathology. While most mouse models are primarily designed with the goal of robustly generating advanced malignancies, in order to study cancer evolution in a more physiological context, one needs the ability to induce mutations in a small number of rare progenitor cells. And since cancer is highly associated with old age, it is crucial to understand how physiological changes related to old age, from immune perturbations to tissue structural changes, impact cancer development and treatments. To begin to understand how contexts such as aging influence the early stages of cancer, our lab has adapted various models to study how tissue microenvironmental changes can impact cancer initiation in the hematopoietic and lung systems. For these studies, we have capitalized on methods which allow direct manipulation of a small fraction of progenitor cells in these tissues, often using viral vectors. Importantly, both the lung and the hematopoietic system are relatively “accessible”. For decades, groups have used retroviral/lentiviral transduction of hematopoietic progenitor cells, followed by transplantation, to initiate leukemia development in mice (e.g. (3)). Similarly, adenoviral and lentiviral vectors have enabled the delivery of CRE recombinase, CRISPR constructs, or cDNAs into the lungs of unconditioned mice, in order to induce oncogenic events and eventually lung cancers (e.g. (4)).

We have made a couple of important modifications to these valuable experimental systems. First, for leukemogenesis, we moved from γ-irradiation-mediated conditioning of mice for transplantation to the use of busulfan, which we have shown is much less inflammatory, does not deplete mature hematopoietic cells, and has only transient effects (5). Thus, busulfan conditioning allows us to study leukemogenesis in a less perturbed microenvironmental context. For oncogenesis in both tissues, we also greatly reduce the fraction of cells that receive the oncogenic event, either by using less virus or by transplanting more unmanipulated cells, which provides for a more competitive context – the oncogenically-initiated cells must prove their worth in relation to healthy competition. Importantly, the viral methods mostly allow for direct induction of oncogenesis in wild-type mice, which is particularly convenient for the study of aging (obviating the need to maintain genetically modified cohorts of mice for a couple of years). For both tissue sites, the methods available to us (developed by other groups) facilitated the development of models to study oncogenesis in physiological tissue environments.

We have used these methods to demonstrate that insults including aging and radiation exposure within the bone marrow microenvironment can promote the selection of oncogenic events that become adaptive in the altered tissue microenvironment. We have shown that B cell progenitors in aged mice exhibit signaling and metabolic defects, which can then contribute to the selection of oncogenes such as BCR-ABL and mutant NRAS that restore fitness defects in B progenitors, thereby, increasing leukemogenesis (5). Importantly, we have demonstrated that reducing inflammation can prevent the aging-associated decline in B progenitor fitness, obviating selection for adaptive oncogenic events. Thus, dampening one component of this aged landscape, inflammation, can prevent oncogenic adaptation, highlighting the critical role for aging-altered tissue landscapes in dictating whether or not an oncogenic mutation is positively selected. While we cannot avoid the occurrence of many mutations as we age, these results indicate that we can change the evolutionary trajectories of potentially oncogenic mutations. Additional studies have demonstrated how prior radiation exposure impairs hematopoietic stem cell fitness by reducing self-renewal, thereby selecting for oncogenic events like CEBPA loss that restore self-renewal (6).

While our studies examining the role of aging and cigarette smoking for oncogenesis in the lung are ongoing, we have gained valuable lessons from our leukemogenesis studies that guide our lung research. In particular, we have developed methods to analyze and manipulate inflammation, and to characterize leukocytic populations which contribute to the microenvironments in both tissues. We also learned critical lessons from the leukemogenesis models, including the importance of approaches that maintain a normal tissue landscape (e.g. avoiding harsh conditioning), that oncogenesis should be modeled through initiation in rare cells, that aged mice present far more inter-animal variability than young mice, as well as the challenges inherent in mechanistically understanding oncogenic adaptation. Moreover, our studies of oncogenesis in the lung have reinforced our focus on the microenvironment, given the more static neighborhood for early carcinogenesis (with an initiated clone surrounded by noncancerous cells). These studies in lung oncogenesis models will be critical for determining whether context-dependent selection for oncogenic events substantially contributes to the role of aging or other insults to promoting cancers in epithelial tissues (from which most cancers are derived).

For the development of novel therapeutics for leukemias and lung cancers, there are valuable models where mice with cancers that evolved in situ or formed by transplantation of cells (either human or mouse) can be treated with novel agents. Our lab has been particularly interested in understanding immediate cellular adaptation following treatment (not to be confused with *selection* for cells with epigenetic/genetic encoded traits adaptive to the treatment), with the goal of discovering vulnerabilities that can be exploited for therapeutic benefit. Our work in both lung cancer and leukemia models began with shRNA screens to uncover synthetic lethal dependencies engendered by tyrosine kinase inhibition, with a focus on drugs already used in the clinic. Our initial studies were in leukemias, and we transitioned to lung cancer motivated by the great need for new therapies for this disease, which is the number one cancer killer worldwide. Interestingly, the screens in both cancer systems led to similar targets. These studies identified the non-canonical and canonical Wnt pathways as critical for the survival of chronic myeloid leukemia (7) and non-small cell lung cancer cells (8) following treatment with Bcr-Abl and epidermal growth factor receptor inhibitors, respectively. In chronic myeloid leukemia, noncononical Wnt/Ca2+ signaling increased NFAT-dependent IL-4 production providing protection from exposure to Bcr-Abl inhibition (7). In contrast, canonical Wnt signaling mediated through tankyrase contributed to the survival of non-small cell lung cancer (NSCLC) following epidermal growth factor receptor (EGFR) inhibition by impacting β-catenin-dependent transcription (8). Additional screens and follow up studies revealed novel metabolic dependencies for both acute myeloid leukemias and lung cancers (9, 10). Intriguingly, our studies revealed that maintaining redox balance is crucial for the survival of acute myeloid leukemias in the face of FLT3 tyrosine kinase inhibition (9). For lung cancers, EGFR-dependent NSCLC cells rely on the urea cycle to maintain cellular energetics upon EGFR inhibition (10). Critically, our experiences gained in the leukemia realm, both for Wnt signaling and for metabolism, “greased the wheels” for our lung cancer studies. In all cases, the availability of both patient-derived xenograft and syngeneic (immunocompetent) mouse models facilitated the testing of combination therapies where Wnt pathway or metabolic pathway inhibition was shown to improve animal survival under tyrosine kinase inhibitor treatment. In total, these studies have shown how cancers from both sites can immediately adapt to tyrosine kinase inhibition, through Wnt pathways or via metabolic changes, allowing the persistence of a subset of cancer cells and contributing to disease relapse.

Due to the fundamentally different nature of the hematopoietic and lung system, there are also challenges to working in two different organ/cancer systems. For one, due to the vastly different stem cell and tissue organization between the blood and lung, each requires expertise in different techniques and methods. We have relied more heavily on flow cytometry to characterize both normal and malignant hematopoiesis, and on immunofluorescence for similar characterizations of the lung. In addition, while the nature of hematopoietic stem and progenitor cell populations, particularly for the mouse, has been well defined, there is less certainty for the lung, particularly for the identity of cellular populations that serve as the targets for oncogenesis.

Another challenge in working on the hematopoietic and lung system is the ability to keep on top of new scientific findings presented in the literature and at conferences for two fundamentally different cancers/organ systems. Moreover, as our lab is focused on

understanding how changes in normal physiology can contribute to oncogenic adaptation, as well as adaptation following treatments, this requires a vast understanding of two very different realms – cancer initiation and therapeutics. As we are a small lab, it becomes more difficult to technically assist one another as oftentimes particular skills are required for working on either the hematopoietic or lung models. Finally, an argument could also be made that the diverse focuses of a lab dilutes the stature of the investigator in a particular field, as contributions to either leukemia or lung cancer, or to either oncogenesis or therapeutic development, will be less than could be obtained by focusing on one area.

Still, there are additional clear advantages to working in diverse cancer models. As one example, since our lab studies how the context of aging affects normal tissue function and contributes to oncogenic adaptation, we can make better use of our aged mice (for both humane and cost-saving reasons). For analyses of physiological changes in tissues in old age, we regularly isolate and characterize multiple tissues including the lungs and hematopoietic organs from the same mice. We have found that cross-comparisons of results obtained for different tissues from the same sets of mice can be informative, as even mice of the same age show variability in phenotypes. Age-dependent deterioration of the structure, biochemistry or function of one tissue often correlates with similar decline in other tissues from the same mouse, highlighting the systemic nature of aging.

Another advantage presented by both the hematopoietic and lung systems is that there is great potential to translate discoveries, as clinical samples can be obtained relatively easily from both systems. Access to blood and bone marrow facilitates investigations into whether aging-associated perturbations in the blood can inform blood cancer risk and provide insight into leukemia evolution in response to therapy. Since the lung is a “surface tissue”, it is more accessible than a number of other internal organs. Premalignant tissues collected from bronchoscopies and brushings from high risk individuals with smoking history or inflammatory lung diseases can be evaluated for indications of somatic evolution and microenvironmental changes to determine how such changes influence the risk of lung cancer.

Despite the inherent challenges, using these two organ and cancer systems has been invaluable in understanding how cells adapt under specific circumstances, whether engendered by tissue alterations inflicted by exposures and aging or in the context of treatment. For science in general, studying a process in multiple systems can better lead to the establishment of first principles. By studying these two fundamentally different tissues, we hope to be able to develop novel strategies to limit oncogenesis and to improve therapeutic responses, whether through direct targeting of pre-malignant or malignant cells, or by modulating the tissue microenvironment.

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