Zooming in on the cell biology of disease

Rushika M. Perera*
Departments of Anatomy and Pathology and the Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94143

ABSTRACT Today's cell biology could be considered a fusion of disciplines that blends advanced genetics, molecular biology, biochemistry, and engineering to answer fundamental as well as medically relevant scientific questions. Accordingly, our understanding of diseases is greatly aided by an existing vast knowledge base of fundamental cell biology. Gunter Blobel captured this concept when he said, “the tremendous acquisition of basic knowledge will allow a much more rational treatment of cancer, viral infection, degenerative disease and mental disease.” In other words, without cell biology can we truly understand, prevent, or effectively treat a disease?

INTRODUCTION It is an honor to receive the Gunter Blobel Early Career Award from the American Society for Cell Biology. I consider myself a cancer cell biologist, having trained in both cancer and cell biology labs. I have valued the opportunity to merge these two identities over the course of my career in an effort to better understand the fundamental principles that drive cancer cell growth, spread, and resistance to drugs that should normally kill cancer cells. A long-standing interest has been to study these complicated processes at the organelle level. That is, how do changes in the individual parts influence the behavior and fate of the cell as a whole? Cancer, and all its complexities, has served as an exciting, sometimes confusing, but always intriguing “model system” to explore these questions.

A TALE OF TWO WORLDS Just as the study of cell biology increases our ability to understand disease, diseased cells have long served as important tools for uncovering novel cell biology. Consider the myriad of studies on how invading pathogens subvert host cell defenses to establish a symbiotic albeit temporary residence. Understanding how a pathogen is able to rewire a particular circuit can reveal a lot about the circuit itself. Unsurprisingly, the study of invading pathogens has helped to establish basic concepts in endocytosis, membrane trafficking, organelle function, and membrane repair (Asrat et al., 2014; Welch, 2015; Helenius, 2018). Other disease states can also teach us about fundamental cellular programs that maintain organelle homeostasis, nutrient balance, signal transduction, regulated growth, and death.

Many diseases, including cancer, arise from genetic alterations that initiate a chain reaction of events resulting in aberrant cell behavior (Hanahan and Weinberg, 2011; Dancey et al., 2012). To gain a complete picture of how a diseased cell subsequently evolves, it is important to also consider the cell biological changes that arise downstream of a disease mutation. As an honors and graduate student at the University of Melbourne and the Ludwig Institute for Cancer Research in Australia, my first foray into science was to explore how specific genetic mutations in glioblastoma cells—the most lethal cancer of the brain—endows them with extraordinary growth and survival capabilities, while simultaneously imposing unique vulnerabilities that can be exploited therapeutically. Working on a project that had clear implications for how patients might be treated in the future was particularly appealing to me as a young trainee. I distinctly remember observing how treatment of mice with experimental therapies targeting cancer-specific mutant proteins led to a dramatic shrinkage of tumors over time. However, I also noticed that some drugs didn’t have the same antigrowth effects despite being closely...
related to the more effective drug. At that time, I didn’t know how to “zoom in” and uncover what was happening within a cell. This ignited a curiosity that would take me across the world to study how cells function.

I was fortunate to spend part of my graduate studies at Yale University in the laboratories of Derek Toomre and Pietro De Camilli (winner of this year’s E. B. Wilson Medal). It is here that I discovered the power of cell biology and live cell microscopy and learned how to piece together the inner workings of the cell. Pietro and Derek’s labs were pioneering state-of-the-art methods to track endocytosis in living cells in real time—an endeavor that was of particular interest to many in the cell biology community at the time. Our specific goal was to study how dynamic recruitment of key lipid kinases and phosphatases to the plasma membrane reshapes its phosphoinositide composition, which in turn orchestrates the many interlocking steps of endocytosis, subsequent intracellular trafficking, and the signaling intensity of growth factor receptors. As with my experiences observing tumors shrinking before my eyes, I was now observing the inner workings of cells in real time. It was an exhilarating period during which I gained a vast amount of knowledge, learned how to ask good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging good questions, and effectively “grew up” as a scientist. Armed with this new expertise, I felt I was finally ready to tackle that nagging question that remained with me: what makes cancer cells so resilient and stealthy? Are they simply better than a normal cell at executing the same programs, or do they possess fundamentally different programs that have yet to be fully understood? A move to Boston allowed me to explore these ideas and merge my cancer and cell biology worlds to tackle a complicated and deadly disease.

A MERGING OF FIELDS

Pancreatic ductal adenocarcinoma (PDA) is the most lethal cancer of the pancreas and remains one of the biggest challenges in cancer biology and clinical care. In 2021, more than 60,000 patients will be diagnosed with PDA in the United States (Siegel et al., 2021) and, despite important progress in treatment and management, long-term survivors are rare, with only 10% of diagnosed patients surviving 5 years (Siegel et al., 2021). By 2040 PDA will become the second leading cause of cancer-related deaths among men and women (Rahib et al., 2021). As with many diseases, the genetics of PDA is well known—activating mutations in the small GTPase KRAS are near universal in these tumors and trigger a cascade of downstream events that contribute to the aggressive nature of the disease (Ying et al., 2016). In the early 2000s the development of several genetically engineered mouse models of PDA that faithfully recapitulate aspects of the human disease provided an unprecedented ability to study its spatial and temporal evolution in a living host (Aguirre et al., 2003; Hingorani et al., 2003, 2005; Bardeesy et al., 2006; Guerra et al., 2007). From here, studies into the cellular biology of PDA accelerated. I was conducting postdoctoral studies in the lab of Nabeel Bardeesy—a expert in the genetics and biology of PDA—at Massachusetts General Hospital in Boston when two seminal papers published in 2011 by the laboratories of Alec Kimmelman and Eileen White showed that autophagy—a highly conserved intracellular trafficking and recycling pathway—was constitutively activated in KRAS mutant pancreatic and lung cancer cells, respectively (Guo et al., 2011; Yang et al., 2011). Importantly, these studies determined that shutting down autophagy or the lysosome—the essential catabolic organelle that serves as the final step in the autophagy cascade—was sufficient to block tumor growth. From here, new questions arose: how does constitutive activation of the autophagy-lysosome pathway occur in cancer cells, and why is pathway hyper-activation necessary for tumor progression?

Concurrent with these studies, Andrea Ballabio made the critical discovery that activation of autophagy and lysosome biogenesis was a highly regulated process controlled by a family of master transcription factors known as the MIT/TFE proteins (Sardiello et al., 2009; Palmieri et al., 2011; Settembre et al., 2011). This discovery turned on its head the old notion that cellular catabolism occurs constitutively and recast autophagy and lysosomes as a highly integrated system that recognizes and responds to many diverse stimuli and stressors both within and from outside the cell. The eureka moment for me came when I found that the MIT/TFE factors were over-expressed in PDA cells along with a majority of their target genes, which encode key components of lysosomes and the autophagy machinery. I remember bringing my preliminary findings and ideas to Nabeel and explaining what I thought might be uniquely occurring in PDA cells. Nabeel immediately saw the implications of this observation and gave me full freedom and unfettered support to pursue this line of investigation. In this moment my two identities as a cancer biologist and a cell biologist helped me to piece together an unsolved puzzle. In PDA the MIT/TFE factors were constitutively trafficked into the nucleus, leading to hyperactivation of the autophagy-lysosome program. Using electron microscopy, we were also able to show that patient PDA tumors had >10-fold more lysosomes compared with normal pancreas (Perera et al., 2015). Together, these studies zooming in from tumors to cells to organelles were the culmination of a journey to establish a scientific identity of my own and formed the foundation of my future lab.

CANCER CELL BIOLOGY—A FOCUS ON THE LYSOSOME

The advent of new techniques and models often changes the way we study cells and their individual parts. Along with two-dimensional cultured cells, we now have access to patient-derived organoid cultures and tissue specimens; the arrival of CRISPR has made the generation of genetically engineered animal models faster and more efficient; major leaps in genetic sequencing and profiling at the single-cell level has uncovered new cellular states and transitions and improved methods to visualize, purify, and profile organelles as uncharted new features and functions linked to a variety of cellular contexts. My lab has utilized methods to rapidly purify our organelle of choice—the lysosome—to answer a simple yet fundamental question: are lysosomes in cancer cells different from those in normal cells? Through this endeavor, we have uncovered how the lysosome plays a central role in diverse cellular processes from regulation of cancer cell metabolism to escape from immune cell-mediated killing as well as the great lengths taken to protect this key organelle against damage and dysfunction (Yamamoto et al., 2020; Gupta et al., 2021). Through collaboration with colleagues, we have helped to uncover how autophagy and lysosomes contribute to cellular differentiation, exosome biogenesis, and nutrient sensing (Althuizer-Keylin et al., 2016; Castellano et al., 2017; Kilinc et al., 2021). I believe that we have only scratched the surface when it comes to understanding lysosome function, composition, biogenesis, and interaction with other organelles in healthy and diseased states. I am excited to continue this exploration with the hope that our collective discoveries will unearth new concepts in cell biology and also inform new strategies for combating deadly diseases.

ACKNOWLEDGMENTS

I am forever grateful to my mentors—Terrance G. Johns, Andrew M. Scott, Derek K. Toomre, Pietro De Camilli, Ira Mellman, Jeffrey Settembre, Nabeel Bardeesy—for their support and encouragement. Thank you to a wonderful group of colleagues at the University of California, San Francisco, who have made the transition to PI a far
REFERENCES

Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 17, 3112–3126.

Altschuler-Keylin S, Shinoda K, Hasegawa Y, Ikeda K, Hong H, Kang Q, Yang Y, Perera RM, Debnath J, Kajimura S (2016). Beige adipocyte maintenance is regulated by autophagy-induced mitochondrial clearance. Cell Metab 24, 402–419.

Asrat S, de Jesus DA, Hempstead AD, Ramabhadran V, Isberg RR (2014). Bacterial pathogen manipulation of host membrane trafficking. Annu Rev Cell Dev Biol 30, 79–109.

Bardeesy N, Cheng KH, Berger JH, Chu GC, Pahler J, Olson P, Hezel AF, Horner J, Lauwers GY, Hanahan D, DePinho RA (2006). Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev 20, 3130–3146.

Castellano BM, Thelen AM, Moldavski O, Feltes M, van der Welle RE, Mydock-McGrane L, Jiang X, van Eijkeren RJ, Davis OB, Louie SM, DePinho RA (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 17, 3112–3126.

Petera RM, Stoykova S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, Lengrand J, Deshpande V, Semig MK, Ferrone CR, et al. (2015). Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. Nature 524, 361–365.

Rahib L, Wehner MR, Matrisian LM, Nead KT (2021). Estimated projection of US cancer incidence and death to 2040. J Am Med Assoc Netw Open 4, e214708.

Sardiello M, Palmeri M, di Ronza M, Medina DL, Valenza M, Gennari VA, Di Malta C, Donaudy F, Embrone V, Polisbukh RS, et al. (2009). A gene network regulating lysosomal biogenesis and function. Science 325, 473–477.

Settembre C, Di Malta C, Polito VA, Garcia Arecibiza M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, et al. (2011). TFEB links autophagy to lysosomal biogenesis. Science 332, 1429–1433.

Siegel RL, Miller KD, Fuchs HE, Jemal A (2021). Cancer statistics, 2021. CA Cancer J Clin 71, 7–33.

Welch MD (2015). Why should cell biologists study microbial pathogens? Mol Biol Cell 26, 4295–4301.

Yamamoto K, Venida A, Yano J, Biancur DE, Kiuchi M, Gupta S, Sohn ASW, Mukhopadhyay S, Lin EY, Parker SJ, et al. (2020). Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. Nature 581, 100–105.

Yang S, Wang X, Contin G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell’antonio G, et al. (2011). Pancreatic cancers require autophagy for tumor growth. Genes Dev 25, 460–470.

Volume 32 December 1, 2021 Zooming in on the cell biology of disease | 3