Zooming in on the cell biology of disease

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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?**

Monitoring Editor
Matthew Welch
University of California, Berkeley

Received: Sep 23, 2021
Revised: Oct 5, 2021
Accepted: Oct 5, 2021

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.

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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

DOI:10.1091/mbc.E21-09-0459
Rushika M. Perera is the recipient of the 2021 Gunter Blobel Early Career Award.
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Abbreviations used: CRISPR, clustered regularly interspaced short palindromic repeats; MiT/TFE, microphthalmia/transcription factor E; PDA, pancreatic ductal adenocarcinoma; PI, principlal investigator.

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 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—an 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 hyperactivation 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 overexpressed 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 unearthed 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 (Altshuler-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 Settleman, 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

less painful one, especially Zena Werb—my former lab neighbor and faculty mentor, whom we dearly miss. To my colleagues, collaborators, and friends across several scientific communities—thank you for welcoming and supporting me over the years. I feel very fortunate to work alongside a talented and motivated group of young scientists in my lab who manage to always keep me on my toes. Finally, to my family, whom I left behind in Australia so long ago, and my wonderful spouse—thank you for your generosity, love, and support. Research in the Perera lab is generously supported by grants from the National Institute of Health/National Cancer Institute, the Damon Runyon Cancer Research Foundation, the Pancreatic Cancer Action Network/American Association for Cancer Research, the Hirshberg Foundation for Pancreatic Cancer, and the National Science Foundation.

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.

Altshuler-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, *et al.* (2017). Lysosomal cholesterol activates mTORC1 via an SLC38A9-Niemann-Pick C1 signaling complex. Science 355, 1306–1311.

Dancey JE, Bedard PL, Onetto N, Hudson TJ (2012). The genetic basis for cancer treatment decisions. Cell 148, 409–420.

Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP, Barbacid M (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 11, 291–302.

Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, *et al.* (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 25, 460–470.

Gupta S, Yano J, Mercier V, Htwe HH, Shin HR, Rademaker G, Cakir Z, Ituarte T, Wen KW, Kim GE, *et al.* (2021). Lysosomal retargeting of myoferlin mitigates membrane stress to enable pancreatic cancer growth. Nat Cell Biol 23, 232–242.

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. Cell 144, 646–674.

Helenius A (2018). Virus entry: looking back and moving forward. J Mol Biol 430, 1853–1862.

Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, *et al.* (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 4, 437–450.

Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA (2005). Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell 7, 469–483.

Kilinc S, Paisner R, Camarda R, Gupta S, Momcilovic O, Kohnz RA, Avsaroglu B, L'Etoile ND, Perera RM, Nomura DK, Goga A (2021). Oncogene-regulated release of extracellular vesicles. Dev Cell 56, 1989–2006.e6.

Palmieri M, Impey S, Kang H, di Ronza A, Pelz C, Sardiello M, Ballabio A (2011). Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. Hum Mol Genet 20, 3852–3866.

Perera RM, Stoykova S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, Lengrand J, Deshpande V, Selig 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, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS, *et al.* (2009). A gene network regulating lysosomal biogenesis and function. Science 325, 473–477.

Settembre C, Di Malta C, Polito VA, Garcia Arencibia 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, Kakiuchi 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, Contino 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, 717–729.

Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, DePinho RA (2016). Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 30, 355–385.