The thrill of scientific discovery and leadership with my group

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ABSTRACT My group and I feel tremendously honored to be recognized with the 2016 Early Career Life Scientist Award from the American Society for Cell Biology. In this essay I share the scientific questions that my lab has been excitedly pursuing since starting in August 2009 and the leadership behaviors we have adopted that enable our collective scientific productivity.

MY LAB'S SCIENTIFIC CONTRIBUTIONS AND VISION

My lab is fascinated by understanding how tissues renew themselves throughout an organism's lifetime. Specifically, we are interested in understanding how cells orchestrate tissue growth and make cell-fate choices that result in balanced tissue regeneration. Tissue regeneration is often looked at as a process happening in a vacuum: each individual cell is an actor in a play, enacting its role according to an unchanging "script." Yet continuous insults, such as tissue tear and somatic mutations, among others, can create continuous variations to the "script." Thus, since these variations may call for improvisation, cells must adapt their roles to keep a tissue (play) functional (entertaining). As I started my lab, I felt that the biggest challenge in understanding mammalian tissue regeneration was that the field largely used static analyses that prevent the ability to capture cells in action in the context of an intact organism in which variations occur. During my doctoral thesis, I experi-enced firsthand how live imaging provided me not only a better understanding of tissue patterning but also allowed us to discover new biology that we had not anticipated, whereby epithelial cells secrete vesicles (called argosomes) that carry morphogens and disperse them throughout the tissue (Greco et al., 2001).

Thus, as I set up my lab, we studied tissue regeneration by investing in a high-risk/high-reward approach to establish skin stem cell imaging in live mice. After more than one year of trouble-shooting and several discouraging road-blocks, we were finally able to visualize, track, and manipulate stem cells and their niches within the skin epithelium of an intact living mouse (Rompolas et al., 2012; Pineda, Park, et al., 2015). These novel approaches have allowed us to get a fresh look at processes that have been investigated for decades, leading to the capture of novel principles of stem cell biology and tissue regeneration. In retrospect, what I had accomplished was combining my passion for visualizing biological processes in vivo with my knowledge of stem cells gained during my postdoc (Greco, Chen, et al., 2009). The ability to directly observe a biological phenomenon is the reason why I fell in love with science, and I was able to bring this angle to bear on our research, which uniquely poised my lab to address previously inaccessible questions and distinguished my lab from the laboratories of my previous mentors (Park et al., 2016).

With these tools, my lab has contributed to the understanding of fundamental principles of the equilibrium of cell choices reached during tissue regeneration and has explored the edges of this equilibrium, which we describe in more detail below. Questions that

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fueled our science include, What are the rules that sustain robust daily tissue regeneration? How many ways are there to ensure tissue function? How does this equilibrium evolve when the normal tissue is in the presence of cancerous proliferative clones?

The niche's requirement for stem cell fate

Tissue regeneration is achieved through a balance of cell production (growth) and elimination (regression). Yet we still fail to understand how stem cells and their environment balance tissue growth and regression during regeneration in a live mammal. To address these questions, we used the mouse hair follicle, which cycles between these phases while maintaining a pool of stem cells to sustain tissue regeneration. By visualizing stem cell behavior and manipulating stem cells' niche during growth, we have shown that 1) stem cell fate depends on the position (surrounding niche) that the cells inhabit, and 2) while the niche is required for tissue regeneration, dedicated stem cells are dispensable. Specifically, we showed that cells can switch their fate to adopt new stem cell functions in the face of loss of a specific stem cell pool. These discoveries have provided a new understanding of the role of stem cell location and how fundamentally important the native/local niche is with respect to stem cell decisions and overall tissue regeneration, which could not have been observed without our ability to track the same cells over time in a live mammal. Our results also reveal a robust mechanism of compensation in which cells from other epithelial compartments can adopt new stem cell functions and fuel tissue regeneration (Rompolas *et al.*, 2012, 2013). We have shown that hair follicle epithelial stem cells are eliminated during regression through a spatial gradient of apoptosis along the same axis utilized for growth. Furthermore, we have demonstrated that hair follicle stem cells collectively act as phagocytes to clear dying epithelial neighbors. Through cellular and genetic ablation, we have shown that epithelial cell death is extrinsically regulated by the local niche through transforming growth factor (TGF)-β activation. Strikingly, our data show that regression acts to reduce the stem cell pool, and the inhibition of the regression phase results in excess basal epithelial cells with regenerative abilities (Mesa *et al.*, 2015). These findings are surprising, because the field previously thought of stem cells as having a finite lifetime/capacity of divisions that eventually leads to their elimination by exhaustion. Indeed, this work shifts the understanding to the niche environment, which, if altered, can lead to the aberrant coopting of the system toward deregulated growth. It also demonstrates that reinstalling a proper niche can correct stem cell–driven aberrancy. This principle is key to TGFβ-driven cancer models and, importantly, also elevates the significance of the niche in the broader study of mechanisms of cancer initiation.

Currently, we are addressing how these cellular interactions are regulated by surrounding niche populations such as mesenchymal cells and immune cells and structural elements such as the extracellular matrix. Together, these approaches will allow us to determine whether specific niche populations that are interspersed or adjacent to the epithelium serve as regional checkpoints to locally control the regeneration process. Additionally, this work has allowed us to branch out and begin to study how cells ensure rapid tissue repair after injury. We have been able to study how the interplay between repair behaviors, such as migration and proliferation, lead to effective reconstruction of the epithelial tissue and the extent to which homeostatic processes such as differentiation are affected during the repair process. We are particularly excited to study how these interconnected cellular behaviors contribute to the tissue-scale changes observed in the repair process.

Individual and group stem cell behaviors in normal and cancerous tissues

Tissue regeneration relies on a multitude of distinct cellular behaviors. Yet we lack an understanding of how these individual cellular behaviors are regulated and how mutations may influence them. To answer these questions, we have traced the entire lifetime of epidermal stem cells and interrogated their behaviors. We have demonstrated that stem cells do not appear to be intrinsically biased toward either self-renewal or differentiation but instead seem to be influenced by the behaviors of their neighboring sister cells. Additionally, as basal stem cells stochastically commit to differentiation, they reuse existing structural organizations (Rompolas, Mesa, *et al.*, 2016; Xin *et al.*, 2016). Recent studies have reported that morphologically normal skin often carries oncogenic mutations. We therefore began to interrogate the interface between normal tissue and cancerous clones by using the evolutionarily conserved pathway, Wnt/β-catenin. Our efforts uncovered a novel mechanism of action for β-catenin that acts non–cell autonomously within the hair follicle stem cells by recruiting wild-type cells to induce de novo hair growth that ultimately results in tumors. Additionally, we show that β-catenin–driven growth is triggered and expands independent of the local niche's influence (Deschene, Myung, *et al.*, 2014). This work changes our understanding of how cells that carry mutations can interact with neighboring cells, providing insights into how cancer initiation and progression may be fueled.

To understand how to counterbalance cancerous growth, we took advantage of two contrasting mouse cancer models: a unique, benign skin tumor that regresses spontaneously, keratoacanthoma; and a malignant skin tumor, squamous cell carcinoma (SCC). We demonstrated that self-regressing keratoacanthoma tumors counterbalance excessive proliferation by employing a homeostatic mechanism of terminal differentiation to regress. When this differentiation cue, retinoic acid, is used on SCC it could also induce the regression of these malignant tumors (Zito *et al.*, 2014). Taken together, this body of work modifies prevailing views in the field regarding how cells that carry mutations can interact with neighboring cells, expanding our understanding of how tumor progression and regression is regulated.

Currently, we are interested in mutations associated with SCC, such as clones bearing *Hras* mutations in combination with a loss of TGFβ function. Thus, we are using live imaging to study the dynamic behaviors and interactions between mutant clones (double and single mutants) and wild-type neighboring tissues. Functional investigation of both oncogenic signaling pathways and different cellular interactions will help us elucidate the critical set of decisions that lead to cancer.

RESPONSIVE LEADERSHIP: ADAPTING TO MY GROUP TO GENERATE SCIENTIFIC DISCOVERIES

During my journey, I have often reflected on the "features" of a successful leader and will explain below the thought process that brought me to evolve a model of leadership that is based on the complementary strengths and weaknesses of all who work in the lab. In searching for my voice in a leadership role, I encountered a conundrum. The search for leadership "features" revolved around the principal investigator (PI) as the *leader*. While the PI is fundamental in the initial phases of a lab—the founder who needs to begin the lab with a great idea—as the group expands in size over time, the lab's effectiveness increasingly depends the vision of all of its constituents, not just the PI. Yet models of leadership I have been exposed to, from grant preparation to promotion to credit in papers, seemed to single out one element in this collective project,

FIGURE 1: Greco lab—annual retreat August 2016. From left to right, top row: Kai Mesa, Katie Cockburn, Jonathan Boucher, Cristiana Pineda, David Gonzalez, Tianchi Xin, Eduard Marsh, and Samara Brown. Second row: Sangbum Park, Valentina Greco, and Catherine Martone.

the PI, but didn't take into account that the members of the group are also a critical part of the leadership equation (Berg, 1998). As a new PI, I faced a number of challenges implementing a model of leadership in my lab. One such challenge was that I didn't recognize in myself what society seems to value as innate successful features of a leader, in that I felt at a disadvantage being a woman and an immigrant. Historically, these characteristics are (consciously or unconsciously) not often associated with people in positions of leadership in the United States. I am proud to be a woman and an immigrant. At times these characteristics posed unique challenges that caused me to struggle in my career. But hard work as well as support from my lab, my peers, and a handful of senior colleagues has made scientific and personal success possible and rewarding. Over the years, in speaking with others in positions of leadership, I have found that, encouragingly, several models were available as alternatives to the trait theory of leadership. These alternatives point to a view of leadership as not something we are born with but rather a diverse set of behaviors and acquired skills. I was then faced with identifying what successful behaviors a person in a leadership role should adopt. While our society seems drawn to behaviors such as self-confidence, assertiveness, and strength, these struck me also as *individual* behaviors not necessarily connected to the groups with which we work. In fact, leadership is about the effective work of a collective group, and those behaviors seem to have little to do with the group itself. I finally came to realize that, in my view, the most successful behaviors that people in positions of leadership would need to adopt would *evolve around the demands of the group.*

Thus, in my view, this requires people in positions of leadership to acquire at least two essential behaviors: first, knowing one's own weaknesses, and second, effectively adapting to the group by listening to feedback and developing effective solutions to the problems raised.

Harnessing one's weaknesses

I believe that our society promotes a stereotype that people in positions of leadership are infallible and superior to most of the people around them ("Isn't that how they got to the top?"). In my opinion, this myth reinforces the distance between the PI and the lab members, therefore diminishing the effectiveness of the group's productivity. However, I have also found that recognizing one's limitations is not always simple. While we may be able to get as far as diagnosing our limitations, I felt resistance in myself and in my colleagues when it came to admitting to those limitations and reaching out for help from the group. One hypothesis may be that we fear our disclosure of weakness or limitation will result in our lab members losing trust in us. After growing comfortable with admitting my limitations and feeling vulnerable in front of my lab, I now work with my group and the individual lab members to compensate for my weaknesses (these range from being a procrastinator on specific tasks that don't come naturally to me to merely admitting that, as a single individual, I have a biased and narrower view on any subject under discussion, despite many years of education and the title of associate professor). As I share my weaknesses, I also engage my lab members in compensating for them (and complementing my strengths) by working together on tasks and creating a better outcome on all fronts. Thus, my weaknesses turn into a positive tool that empowers my lab members and allows them to grow better and faster, preparing them for future roles in positions of leadership while building a more cohesive group in which everyone is valued.

Establishing a feedback model

I would not be where I am today were it not for the insightful and invaluable contributions of each person in my lab (Figure 1). Paradoxically, that also places me in a position of vulnerability. I have always felt that we use more reductionist approaches in viewing people, ranking the first and seeing the rest as less valuable. I have found that it takes courage, time, but most importantly, genuine belief to view everyone we have hired within an organization, some of whom fall "below" in a formal hierarchical structure, as significant contributors. I have come to realize that this belief in the meaningful contribution of all members of the lab is a fundamental component of a successful group. Therefore, I work hard to maintain a culture in my lab in which feedback from lab members is not only encouraged but listened to carefully and very often leads to changes that deeply affect the course of our lab decisions. While it has not always been easy and requires a significant investment of time and a willingness to accept that the PI's ideas are not always welcomed with open arms by the lab members, it has created an evolving entity that empowers each individual within our small organization.

I believe an obstacle to establishing this model comes from the fear we carry of not feeling adequate. I believe this feeling is shared within any profession in which present and future performance is what determines our own and our peers' appreciation independent of previous accomplishments. A mentee once asked me what happens if a lab member is "better" than the PI. This thought was posed to me with the assumption that such a lab member is a potential threat. I started to think that people feel a need to establish a hierarchical scale to rank people from best to worst (similar to the

ranking of the traits I was discussing earlier). In a separate conversation with a colleague, I was told that I needed, once in a while, to establish the superiority of the PI over the lab members. My hypothesis is that both conversations were stemming from this fear we carry of becoming dispensable, which in turn may trigger a dominant, repressing behavior in us. Thus, this repression is not based on actual superiority but on fear. Regardless, these behaviors that our group will silently watch and learn from will inhibit a transparent critical dialogue, which in turn limits the power of the lab for discovery. If we instead foster a system in which 1) each individual is valued for his or her strengths *and* weaknesses (one person's weaknesses leave room for the contributions of others) and 2) the PI is committed to soliciting and responding to lab members' feedback on important lab decisions, I believe we create the opportunity for significant scientific achievement.

I believe that these reflections on leadership could be applied to any group setting and therefore to any person working in a position of leadership. Thus, our scientific community at large could profit from a similar model, wherein more engagement of our members, seeing them as accountable and driving forces of the group itself, may accelerate both individual and collective growth.

REFERENCES

Boldface names denote co–first authors.

Berg DN (1998). Resurrecting the muse: followership in organization. In: The Psychodynamics of Leadership, ed. EB Klein, FG Gabelnick, and P Herr, Madison, CT: Psychosocial Press.

Deschene RE, Myung P, Rompolas P, Zito G, Sun TY, Taketo MM, Saotome I, Greco V (2014). β-catenin activation regulates tissue growth via a non-cell autonomous mechanism within the hair stem cell niche. Science 343, 1353–1356.

Greco V, Chen T, Rendl M, Pasolli A, Stokes N, Fuchs E (2009). A two-step mechanism for stem cell activation during hair regeneration. Cell Stem Cell 4, 155–169.

Greco V, Hannus M, Eaton S (2001). Argosomes: a potential vehicle for the spread of morphogens through epithelia. Cell 106, 633–645.

Mesa KR, Rompolas P, Greco V (2015). The dynamic duo: niche/stem cell interdependency. Stem Cell Rep 4, 961–966.

Park S, Greco V, Cockburn K (2016). Live imaging of stem cells: answering old questions and raising new ones. Curr Opin Cell Biol 43, 30–37.

Pineda CM, Park S, Mesa KR, Wolfel M, Gonzalez D, Haberman A, Rompolas P, Greco V (2015). Intravital imaging of hair follicle regeneration in the mouse. Nat Protocols 10, 1116–1130.

Rompolas P, Deschene ER, Zito G, Gonzalez DG, Saotome I, Haberman AM, Greco V (2012). Live imaging of stem cell and progeny behavior in physiological hair follicle regeneration. Nature 487, 496–499.

Rompolas P, Mesa KR, Greco V (2013). Spatial organization within a niche as a determinant of stem cell fate. Nature 502, 513–518.

Rompolas P, Mesa KR, Kawaguchi K, Park S, Gonzalez D, Brown S, Boucher J, Klein AM, Greco V (2016). Spatiotemporal coordination of stem cell commitment during epidermal homeostasis. Science 352, 1471–1474.

Xin T, Greco V, Myung P (2016). Hardwiring stem cell communication through tissue structure. Cell 64, 1212–1225.

Zito G, Saotome I, Liu Z, Ferro EG, Sun TY, Nguyen DX, Bilguvar K, Ko CJ, Greco V (2014). Spontaneous tumor regression in keratoacanthomas is driven by Wnt/retinoic acid signaling cross-talk. Nat Commun 5, 3543.