ESSAY

SCIENCE & SCILIFELAB PRIZE ESSAY

Teamwork: The tumor cell edition

Subclone cooperation maintains tumor growth

By **Allison S. Cleary**

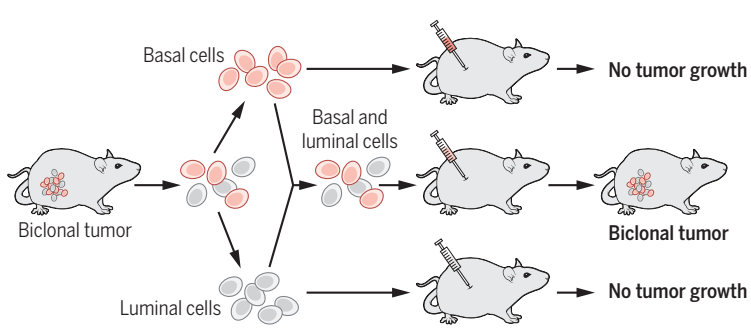
A remarkable degree of heterogeneity exists within individual breast cancers. Indeed, intratumoral heterogeneity has been appreciated since the 19th century, when Rudolf Virchow and other early pathologists noted the morphologic heterogeneity among individual tumor cells. More recently, cancer genome–sequencing studies have revealed the presence of multiple genetically distinct tumor cell populations, termed subclones, coexisting within individual breast cancers (*1*–*5*). This intratumor heterogeneity poses significant challenges in treatment efforts, but it also raises interesting questions about the nature of tumor progression.

The leading theory that attempts to explain genetic subclonal diversity within tumors is the clonal evolution theory that applies the principles of Darwinian evolution to expanding tumor cell populations (*6*). As such, individual subclones are often depicted as self-interested competitors in a battle for the position as the dominant, or "fittest," tumor cell clone. However, in nature, the "fitness" of a given species often depends upon its ability to interact and cooperate with others in its environment. If we consider genetically distinct tumor cell subclones as discrete species within the tumor microecosystem (*7*), would those same ecological principles apply? In fact, could the genetic heterogeneity within individual breast cancers be a cause, rather than a consequence, of clonal evolution and tumor progression?

As a graduate student in Edward Gunther's laboratory at the Pennsylvania State University College of Medicine, I became fascinated with the idea that this commonly observed subclonal diversity might have a functional role in breast tumor development and progression. For my graduate dissertation project, I chose to investigate this idea further using the classic MMTV-Wnt1 mouse mammary tumor model (*8*). In this model, mammary-specific expression of the Wnt1 oncogene is known to produce tumors with a mixed-lineage histology: that is, the tumors consist of both luminal and basal epithelial cell populations. Secreted Wnt1 protein is produced exclusively by the luminal epithelial population and interacts with the basal cell population through short-range paracrine signals (*9*). The mixed-lineage character of these tumors was generally thought to derive from a hierarchical organization, in which tumors originated from a common progenitor cell whose progeny were capable of differentiating into both of the component epithelial lineages (*9*–*12*). Thus, although exhibiting apparent cellular heterogeneity, all of the tumor cells were thought to represent a single clone.

Using somatic mutations in the *HRas* oncogene as a marker for clonality, we found that some tumors did indeed conform to a hierarchical organization and displayed identical *HRas* mutations in both the luminal and basal tumor cell subsets. Yet, for other tumors, mutations in *HRas* were detectable only within the basal epithelial compartment. Conversely, *Wnt1* expression, as determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and set (as high or low) relative to an unsorted sample, was markedly enriched within the luminal subset. Consequently, these tumors contained at least two genetically distinct subclones: one composed of $Wnt1^{high}$ $HRas^{wild\text{-}type}$ luminal cells and another composed of $Wnt1^{low}$ $HRas^{mutant}$ basal cells (*13*). What's more, this apparent biclonality proved to be a stable property within the tumors. Notably, after isolating each of the subclones, we found that neither was capable of propagating tumor growth alone. An admixture of the two populations, however, was found to be extremely tumorigenic, which suggested a cooperative relationship existed between the two subclonal groups (see the figure).

Next, we wondered what would happen if that cooperative interaction was interrupted. We knew that tumor growth for this model strictly depended upon continued Wnt1 signaling. But, if the cells were deprived of that signal, could tumor growth be rescued by providing access to an alternate source of Wnt1? We hypothesized that, if these tumors were truly biclonal and dependent upon a cooperative interaction between the two subclones, then the cells might be able to reestablish a similarly interdependent relationship with an unrelated population of cells—for instance, one derived from a completely separate animal. To address this question, we utilized an inducible version of the MMTV-Wnt1 model in which Wnt1 production is contingent upon administration of the small molecule, doxycycline. Biclonal tumors generated in this inducible model were then transplanted into the mammary fat pads of either wild-type host mice or mice in which Wnt1 is continuously expressed. Upon withdrawal of doxycycline, tumors on the wild-type host animals regressed completely. However, after doxycycline withdrawal, the tumors transplanted onto the Wnt1 host animals regressed only partially before exhibiting rapid tumor regrowth. Further molecular analysis revealed that the relapsed tumors were not only biclonal, but chimeric: composed of donor-derived $Wnt1^{low}$ $HRas^{mutant}$ basal cells and host-derived $Wnt1^{high}$ $HRas^{wild\text{-}type}$ luminal cells (*13*). Indeed, these chimeric tumors had recruited Wnt1-producing luminal cells from the surrounding epithelium



Wnt1 tumors with a biclonal organization require both subclones for tumor propagation. Tumors identified as containing genetically distinct luminal $Wnt1^{high}$ $HRas^{wild\text{-}type}$ and basal $Wnt1^{low}$ $HRas^{mutant}$ subclones were separated by fluorescence-activated cell sorting (FACS) into their component cell populations and then transplanted, either separately or as a 1:1 admixture, into mammary fat pads of wild-type host animals. Animals receiving either subclone alone failed to develop tumors, whereas the cell mixture containing both subclones was highly tumorigenic.

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and had incorporated those new cells into the growing tumors, which restored the biclonal cooperative interaction. In fact, many of the chimeric tumor relapses had completely replaced the original luminal cell clone with a new one. Together, these results demonstrated a functional codependence between the distinct tumor cell subclones within these tumors. It also reinforced the idea that there may be a selective advantage for the active preservation of subclonal heterogeneity, in some cases.

Indeed, our study (*13*) was among the first to definitively establish a functional requirement for interclonal cooperation within a spontaneous mammalian tumor model. Since then, several additional studies have described cooperative interactions between various tumor cell subpopulations (*14*–*17*), which suggests that this may be a relatively common mechanism for the maintenance of subclonal diversity. Recent studies have also reported a role for interclonal cooperation in the process of tumor metastasis as well (*18*, *19*).

Ultimately, it has yet to be determined what role interclonal cooperativity plays in human breast cancers. Although normal mammary gland physiology depends heavily upon various paracrine interactions among the diverse populations of cells that make up the mammary ductal epithelium, the degree to which human breast tumor cells maintain comparable paracrine relationships remains unknown. Should interclonal cooperation prove to be an important driver of human breast cancers, it may proffer opportunities for intervention via pharmacologic uncoupling of key interclonal interactions. ■

PHOTOS: (TOP TO BOTTOM) KENNETH SMITH, PENN STATE COLLEGE OF MEDICINE; LUDMIL ALEXANDROV; LIBER ERO FELLOWSHIP PROGRAM; JOHNATHAN ABRAHAM

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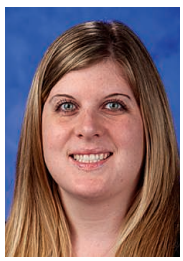
ACKNOWLEDGMENTS

I am extremely grateful to my Ph.D. adviser, E. J. Gunther, for his mentorship. I also thank all of the members of the Gunther lab, both past and present, especially T. L. Leonard and S. A. Gestl.

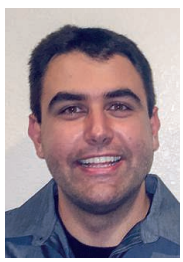
10.1126/science.aad7103



GRAND PRIZE WINNER: CELL AND MOLECULAR BIOLOGY

Allison Cleary

Allison Cleary for her essay "Teamwork: The tumor cell edition." She is originally from Denver, Colorado, and completed her undergraduate degree in Molecular, Cellular, and Developmental Biology at the University of Colorado where she was first introduced to basic science research. From there, she continued her studies at the Pennsylvania State University College of Medicine in their combined M.D., Ph.D. program. While at Penn State, she was fortunate to complete her Ph.D. thesis research in the laboratory of Dr. Edward Gunther, studying mammary gland physiology and breast cancer. Cleary is currently finishing up her M.D. degree and is in the process of applying to Pathology Residency Training Programs on the Physician-Investigator Track. She hopes to be able to continue her work in breast cancer and tumor heterogeneity during this next phase in her training.



CATEGORY WINNERS: GENOMICS AND PROTEOMICS

Ludmil Alexandrov

Ludmil Alexandrov for his essay "Understanding the origins of human cancer." Dr. Alexandrov is an Oppenheimer Fellow in the Theoretical Biology and Biophysics Group at Los Alamos National Laboratory. He earned his Bachelor of Science degree in Computer Science from Neumont University and received his Master's of Philosophy in Computational Biology as well as his Ph.D. in Cancer Genetics from the University of Cambridge. He is a recipient of the 2015 Weintraub Award for Graduate Research and, in 2013, he was listed by Forbes magazine as one of the "30 brightest stars under the age of 30" in the field of Science and Healthcare. His work is focused on understanding the mutational processes responsible for human cancer and human ageing. In 2015, his research was highlighted by the American Society of Clinical Oncology as an important step forward in the fight against cancer.

ECOLOGY AND ENVIRONMENT

Adam T. Ford

Adam T. Ford for his essay "The mechanistic pathways of trophic interactions in human-occupied landscapes." Dr. Ford is a wildlife ecologist interested in how predator-prey interactions are shaped by human-modified landscapes. He received a B.Sc. from the University of Victoria (British Columbia), a M.Sc. from Carleton University (Ontario), and his Ph.D. from the University of British Columbia with Assistant Professor Jacob Goheen. Ford is currently a Liber Ero Postdoctoral Fellow in Conservation Science, based at the Department of Integrative Biology at the University of Guelph (Ontario). The research described in his essay sheds new light on the relationships of people, large carnivores, their herbivore prey, and plants in an East African savanna.

TRANSLATIONAL MEDICINE

Johannes F. Scheid

Johannes F. Scheid for his essay "HIV-specific B cell response in patients with broadly neutralizing serum activity." Growing up in New York and Germany in a family of scientists, Dr. Scheid was fascinated early in life by the career of a physician scientist. During medical school at the Charité University, Berlin, he decided to pursue a Ph.D. at The Rockefeller University. His Ph.D. was based on the observation that some HIV patients develop potent antibodies against HIV. The Ph.D. was awarded the 2012 Harold Weintraub award. After completing medical school and his Ph.D., he worked for 1 year at The Rockefeller University and now continues his clinical training at the Massachusetts General Hospital in Boston.

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Science **350** (6265), 1174-1175.
DOI: 10.1126/science.aad7103