MuSCs with higher PAX3 protein expression that are more prone to proliferate and repair damaged myofibers owing to elevated rates of wear and tear.

Posttranscriptional regulation of stem cell behaviors by microRNAs on cognate mRNAs has been extensively studied in many tissue and organ systems, including skeletal muscle (*10*). The study of de Morree *et al.* demonstrates how simple nongenomic modifications can add an exquisite layer of regulatory control and specific fine-tuning of MuSC function by a third RNA species, U1 snRNA, which affects alternative polyadenylation and thus determines *Pax3* mRNA targeting by miR206. Indeed, it has been shown that more than half of human genes are regulated by alternative polyadenylation, which influences many physiological or pathological processes in various cell types (*11*). Therefore, the intricate interplay between distinct RNA species might have a central role in precise regulation of stem cell behaviors not only in skeletal muscles but also in other tissues and organs. Furthermore, it will be interesting to examine whether similar regulatory mechanisms occur in developmental myogenesis (*5*, *12*).

It has also been well documented that distinct skeletal muscle groups possess differential susceptibility to various types of muscular dystrophies (*13*). For example, in Duchenne muscular dystrophy, clinical symptoms are mainly manifested first in proximal limb muscles in the early teens, followed by respiratory complications involving diaphragm muscles later. This could be associated with differential MuSC activity and muscle turnover in distinct muscle groups mediated by RNA control beyond the genome level. Furthermore, in sarcopenia (age-related muscle loss), RNA control of stem cell fate may also play a role in regulating functional decline of skeletal muscle with age (*14*). Thus, it will be interesting to explore the potential functional relevance of RNA control of MuSCs in the context of development and disease settings in future studies. ■

REFERENCES AND NOTES

1. I. J. Cho *et al.*, *Stem Cell Reports* **12**, 1190 (2019).
2. A. de Moree *et al.*, *Science* **366**, 734 (2019).
3. M. Buckingham, F. Relaix, *Semin. Cell Dev. Biol.* **44**, 115 (2015).
4. F. Relaix *et al.*, *J. Cell Biol.* **172**, 91 (2006).
5. S. C. Boutet *et al.*, *Cell Stem Cell* **10**, 327 (2012).
6. D. Kaida *et al.*, *Nature* **468**, 664 (2010).
7. M. G. Berg *et al.*, *Cell* **150**, 53 (2012).
8. B. Pawlikowski *et al.*, *Skelet. Muscle* **5**, 42-015-0067-1 (2015).
9. A. C. Keefe *et al.*, *Nat. Commun.* **6**, 7087 (2015).
10. A. Shenoy, R. H. Blelloch, *Nat. Rev. Mol. Cell Biol.* **15**, 565 (2014).
11. D. C. Di Giammartino *et al.*, *Mol. Cell* **43**, 853 (2011).
12. K. Goljanek-Whysall *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11936 (2011).
13. A. E. Emery, *Lancet* **359**, 687 (2002).
14. T. Snijders, G. Parise, *Curr. Opin. Clin. Nutr. Metab. Care* **20**, 186 (2017).

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CANCER

Double trouble for cancer gene

Double mutations in an oncogene enhance tumor growth

By **Alex Toker**

Cancer is predominantly a genetic disease. Numerous gain-of-function mutations and gene amplifications that promote cell growth and survival have been identified in human cancer genomes through the efforts of large-scale sequencing projects. One of the most frequently mutated oncogenes in all human cancers is *PIK3CA* [phosphatidylinositol 4,5-bisphosphate (PIP_2) 3-kinase catalytic subunit α], which encodes the catalytic subunit (p110α) of phosphoinositide 3-kinase (PI3K) (*1*). Oncogenic mutations in *PIK3CA* hyperactivate downstream signaling and promote phenotypes associated with malignancy. On page 714 of this issue, Vasan *et al.* (*2*) find that double mutations (two different mutations in one allele) in *PIK3CA* occur with much higher frequency in cancer genomes, particularly breast cancers, than previously thought. Double mutations result in increased PI3K pathway activity and tumor growth and predict increased sensitivity of human breast cancer to PI3K inhibitors.

Small-molecule inhibitors that target the PI3K pathway for cancer therapy have been developed. In May 2019, the U.S. Food and Drug Administration approved alpelisib, a p110α inhibitor for the treatment of postmenopausal women with estrogen receptor–positive (ER^+), *PIK3CA*-mutant advanced or metastatic breast cancer, in combination with the ER antagonist fulvestrant. Approval was prompted by a phase 3 clinical trial that showed doubling of progression-free survival in patients with ER^+ and *PIK3CA*-mutant breast cancer treated with alpelisib and fulvestrant, compared with patients with wild-type *PIK3CA* (*3*). A recent study also identified an exceptional responder (a patient who responded to monotherapy in early clinical trials). This patient had double *PIK3CA* mutations, whereas the majority of recurrent *PIK3CA* mutations identified in sequencing projects were mostly heterozygous, single mutations (*4*).

The enhanced sensitivity to alpelisib was likely due to double mutation in *PIK3CA*, so Vasan *et al.* investigated the pattern and frequency of oncogenic mutations in breast and other cancers. They found that double *PIK3CA* mutations recur in different tumors with much higher frequencies than previously appreciated. Double mutations in *PIK3CA* occurred in 8 to 12% of breast cancer patients with primary as well as metastatic tumors, depending on the cohort analyzed. Double *PIK3CA* mutations were enriched in luminal-subtype ER^+ breast tumors, which have a high frequency of single-hotspot *PIK3CA* mutations (~40%). Additionally, double *PIK3CA* mutations occurred in uterine cancers (27%) and colorectal cancers (12%) and also recur to a lesser extent in numerous other solid tumors.

The three most frequent single-hotspot mutations in *PIK3CA* are $His^{1047}Arg$ in the kinase domain and $Glu^{542}Lys$ or $Glu^{545}Lys$ in the helical domain (*5*). Perhaps surprisingly, the double mutations comprise one of these major hotspot mutations and a second minor site (Glu^{453}, Glu^{726}, or Met^{1043}). Double hotspot mutations—$His^{1047}Arg$ plus $Glu^{542}Lys$ or $Glu^{545}Lys$—were not found, and if they do arise in the cancer cell of origin, presumably they are not selected for during clonal expansion of the primary tumor. The major-plus-minor double mutations occur in the same cancer cell and on the same allele, in cis, as opposed to on different alleles, in trans. Therefore, the resulting mutant p110α protein harbors both mutations as opposed to two separate species of p110α with a different single mutation.

PI3K activation is a complex mechanism that includes engagement of the regulatory p85 subunit of PI3K to phosphotyrosine-containing sequences in upstream receptor tyrosine kinases (RTKs), which in turn relieves the catalytic inhibition of the p110α subunit (*1*). Vasan *et al.* show that the double *PIK3CA* mutations induce PI3K hyperactivation by a combination of disruption of the p85-p110α interaction and enhanced binding of p110α to membranes, where its substrate (PIP_2) is located. The net effect is increased production of the PI3K lipid product and second messenger phosphatidylinositol 3,4,5-triphosphate (PIP_3), which in turn engages downstream effectors, such as the kinase AKT (see the figure).

The consequences of harboring double *PIK3CA* mutations on the same allele are far-reaching. Vasan *et al.* show that double *PIK3CA* mutations lead to significantly increased PI3K activity and downstream path-

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way activation when compared with those of single-hotspot mutations. This results in enhanced tumor growth in vivo. Therapeutically, breast cancer cells with double *PIK3CA* mutations show enhanced sensitivity to alpelisib in vitro and in vivo, compared with that of single-hotspot mutants. Moreover, a retrospective analysis of clinical responses to PI3K inhibitors in breast cancer trials showed that patients with tumors with multiple *PIK3CA* mutations experience a greater overall response to alpelisib as compared with patients with single-mutant tumors.

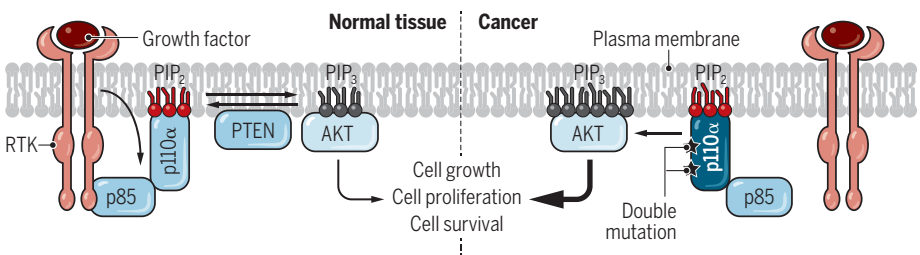
Although single and double mutations in *PIK3CA* are prevalent in some cancers, hyperactivation of the PI3K-AKT pathway is observed in more than 50% of human tumors (*6*). Multiple other genetic alterations in genes that either regulate or transduce PI3K signaling are also frequent. These include amplification or mutations of RTKs, such as members of the epidermal growth factor receptor (EGFR) family, and oncogenic activating mutations or amplification in the three AKT genes: *AKT1*, *AKT2*, and *AKT3* (*6*). Signal termination in the PI3K pathway is achieved primarily through the action of lipid phosphatases, including the tumor suppressor proteins phosphatase and tensin homolog (PTEN), inositol polyphosphate 4-phosphatase type II B (INPP4B), PH domain and leucine-rich repeat protein phosphatase 1 (PHLPP1), and PHLPP2 (*7*). Genetic inactivation of these tumor suppressors in mice leads to enhanced PI3K-AKT signaling and occurs in many human cancers. Thus, genetic mutations in components of the PI3K pathway render it the most frequently mutated pathway in human cancer.

However, single-hotspot mutations in *PIK3CA* are typically insufficient to promote malignancy, and additional "second hit" mutations in cancer-causing genes are required. Vasan *et al.* propose that the presence of double *PIK3CA* mutations in the same allele follows the "oncogene addiction" paradigm (*8*), in which tumors depend on a single gene for malignant transformation and are thus likely to die when the corresponding oncoprotein is therapeutically targeted, whereas single *PIK3CA* hotspot mutations coexist in the same cell and tumor with other PI3K pathway lesions, such as *PTEN* inactivation or *AKT* oncogenic mutations.

These findings are likely to renew interest in the clinical development of PI3K inhibitors. Dose-limiting toxicities and acquired resistance have been noted in patients treated with PI3K inhibitors (*9*), and therefore combination strategies with chemotherapy, immunotherapy, and other targeted agents will likely be most effective. Although alpelisib is potent and highly selective, it is not a p110α-mutant–specific inhibitor, and this may limit efficacy. PI3K inhibitors under clinical evaluation, such as GDC-0077, appear to be selective for mutant p110α (*10*) and therefore may be more effective in patients with double *PIK3CA* mutations. Could double mutations recur in other oncogenes? The approach of Vasan *et al.* could reveal a more complex mutational spectrum in other oncogenes than previously appreciated. ■

Growth factor signaling in normal tissues and cancer

In normal tissues, growth factors activate RTKs, leading to recruitment of PI3K, which converts PIP_2 to PIP_3. This leads to recruitment of downstream effectors, such as AKT, that stimulate cell growth, proliferation, and survival. In cancer, the double mutant *PIK3CA* oncogene encodes hyperactive p110α that is independent of RTK signaling, producing excess PIP_3, which leads to hyperactivation of AKT and uncontrolled cell growth and survival.



PI3K, phosphoinositide 3-kinase; *PIK3CA*, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit α; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase.

REFERENCES AND NOTES

1. D. A. Fruman *et al.*, *Cell* **170**, 605 (2017).
2. N. Vasan *et al.*, *Science* **366**, 714 (2019).
3. F. André *et al.*, *N. Engl. J. Med.* **380**, 1929 (2019).
4. D. Juric *et al.*, *Nature* **518**, 240 (2015).
5. Y. Samuels *et al.*, *Science* **304**, 554 (2004).
6. B. D. Manning, A. Toker, *Cell* **169**, 381 (2017).
7. A. Papa, P. P. Pandolfi, *Biomolecules* **9**, 153 (2019).
8. I. B. Weinstein, A. Joe, *Cancer Res.* **68**, 3077 (2008).
9. A. B. Hanker, V. Kaklamani, C. L. Arteaga, *Cancer Discov.* **9**, 482 (2019).
10. R. Hong, Y.-R. Kao, T.-C. Lee, C.-W. Wu, *Cancer Res.* **78**, 4984 (2018).

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PSYCHOLOGY AND CULTURE

Explaining the puzzle of human diversity

Centuries of Church exposure promote more individualistic and less conforming psychology

By **Michele J. Gelfand**

One of the biggest puzzles facing the social sciences is understanding our immense cultural variation. Over the past several thousand years, humanity has evolved to the point where there now exist 195 countries, more than 7000 languages, and thousands of religions. Research has begun to describe psychological variation across the globe (*1*–*4*), yet only recently have we begun to understand ecological, historical, and sociopolitical factors that produce such differences. Often absent from this mix is how religion and psychological variation are interrelated (*5*, *6*). On page 707 of this issue, Schulz *et al.* (*7*) break new ground in showing how the specific practices of a branch of one of the world's largest religions—Christianity—can in part explain widespread variation in human psychology around the world.

Schulz *et al.* present an intriguing thesis: The Western Catholic Church's Marriage and Family Program (MFP), launched during the Middle Ages (in 506 CE), can partially explain the distinctively individualistic and nonconformist psychology of Western, Educated, Industrialized, Rich, and Democratic (WEIRD) societies in modern times. The MFP radically altered the institution of marriage by prohibiting nuptials within extended families and often requiring newly married couples to set up independent households. Schulz *et al.* predict that longer exposure to the MFP, along with weaker kinship ties that presumably arose from such practices, would drastically alter human psychology, from one that emphasized in-group loyalty, obedience, and conformity, to one that was more individualistic, prosocial toward strangers, and less conforming.

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