As long as large amounts of material were required for dating, its sampling could only be condoned in very rare circumstances. The greatly reduced sample size required for analysis allows subtle removal of small volumes of speleothem directly associated with cave art, from which precise absolute ages may be determined.

Coatings of speleothem calcite do not directly date the cave art itself, but give robust and reliable determinations of the minimum age for the underlying art. Any one calcite overgrowth has a relatively low probability of approaching the age of the underlying art. Studies of overlying calcite layers that consider a small number of examples of cave art in isolation may therefore return ages that do not advance understanding of the age of that art (*2*). By undertaking a broad regional study of many examples of cave art, Pike *et al*. have transcended this problem. The scope of their study has allowed them to unambiguously identify a number of examples that challenge and overturn the previous understanding of that art's origin. In particular, 3 of the 50 examples dated show art to have been created in Spain at around (or indeed possibly before) the time of the arrival of modern humans, bringing current ideas of the prehistory of human art in southern Europe into question.

U-Th dating, particularly of speleothems, has mostly been used to reconstruct climatic change over the past several hundreds of thousands of years (*9*, *10*). The techniques developed for this field now allow archaeological applications to be revisited. Human remains, artifacts, and other evidence of habitation are sometimes coated with calcium carbonate coatings and may thus be dated in the same way as the cave art of Pike *et al*.'s study. Sample material can be collected using micromachining strategies developed for high-resolution climate change chronologies (*6*, *11*), such that growth intervals and durations may be determined, even within a single thin film of carbonate. Other applications include the dating of cave sediments (and any archaeological horizons that they contain) using fragile incorporated fragments of soda straw stalactites (*12*).

All geochronological technologies available for dating prehistorical remains have improved greatly over recent decades. In the case of particularly important or irreplaceable human artifacts, a question has been whether to attempt to date them with available technologies or to wait until less destructive capabilities become available. U-Th dating has become more than 10,000 times more efficient since its invention, and in the case of the technique used by Pike *et al*., there is little room for further spectacular gains. Now allowing cave art and other human artifacts found in caves to be dated based on a few milligrams of speleothem calcite, the U-Th technique can be considered mature enough for widespread use in this field.

Pike *et al*.'s findings push the earliest evidence of cave art in Europe back by several thousand years to at least 40,800 years before the present and raise the question of whether such art was the exclusive domain of modern humans. Further large-scale U-Th studies of cave art in Europe and elsewhere may prove similarly illuminating.

References

1. M. Aubert, *J. Archaeol. Sci.* **39**, 573 (2012).
2. P. Pettitt, A. Pike, *J. Archaeol. Method Theory* **14**, 27 (2007).
3. A. W. G. Pike *et al*., *Science* **336**, 1409 (2012).
4. R. L. Edwards, J. H. Chen, G. J. Wasserburg, *Earth Planet. Sci. Lett.* **81**, 175 (1987).
5. J. Hellstrom, *J. Anal. At. Spectrom.* **18**, 1346 (2003).
6. D. L. Hoffmann, C. Spötl, A. Mangini, *Chem. Geol.* **259**, 253 (2009).
7. P. S. C. Taçon *et al*., *J. Archaeol. Sci.* **39**, 492 (2012).
8. A. W. G. Pike *et al*., *J. Archaeol. Sci.* **32**, 1649 (2005).
9. H. Cheng *et al*., *Science* **326**, 248 (2009).
10. R. N. Drysdale *et al*., *Science* **325**, 1527 (2009).
11. R. N. Drysdale *et al*., *Geology* **35**, 77 (2007).
12. E. St Pierre, J.-X. Zhao, Y.-X. Feng, E. Reed, *J. Archaeol. Sci.* **39**, 922 (2012).

10.1126/science.1224185

CANCER

Cancer and Telomeres—An ALTernative to Telomerase

Finding ways to target the alternative (ALT) telomere lengthening pathway found in some cancer cells could complement telomerase inhibitors currently in clinical trials.

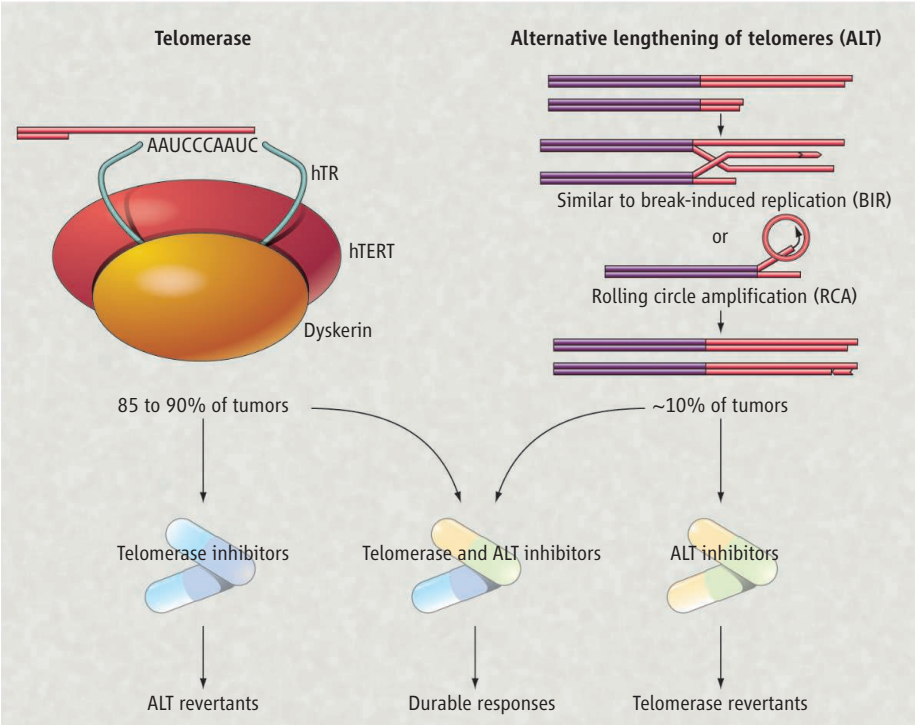
Jerry W. Shay,[1] Roger R. Reddel,[2] Woodring E. Wright[1]

To grow indefinitely, human cancer cells must counteract the progressive loss of telomeric DNA that universally accompanies cell division. To do this, about 85 to 90% of cancers use telomerase (*1*–*3*), an enzyme that synthesizes the tandem 5′-TTAGGG-3′ hexanucleotide repeats of telomeric DNA by reverse transcription using its own RNA subunit as a template. Because telomerase is not expressed in most normal human cells, telomerase inhibition is considered an almost universal oncology target, and several clinical trials are under way (*4*). However, the future success of inhibiting telomerase to treat cancer is far from assured. Indeed, ~10 to 15% of human cancers lack detectable telomerase activity (*3*), and many of these use an alternative lengthening of telomeres (ALT) mechanism (*5*). These ALT-expressing tumors would not be expected to respond to anti-telomerase therapies, and telomerase-expressing tumors could become resistant by switching to an ALT mechanism, as has recently been shown in mice (*6*). Here we present a brief history of ALT research, outline what is left to learn about the pathway, and propose it as a valuable drug target both alone and in combination with telomerase as a dual-targeted anticancer strategy.

Because of the semiconservative mechanism whereby DNA is replicated, all cellular proliferation is accompanied by progressive telomere shortening. When telomeres become short, they elicit a DNA damage response that leads to the irreversible cell cycle arrest known as replicative senescence, which is a potent anticancer protection mechanism (*7*). Occasionally human cells acquire mutations that permit bypass of senescence to prolong proliferation. This results in telomeres that are so short that chromosome ends fuse together, leading to increased genomic instability, aneuploidy, and cell death. Rarely, the ensuing genomic changes result in engagement of a telomere maintenance mechanism (telomerase or ALT) and therefore unlimited proliferative potential, which contributes indirectly to the development of cancer (*7*).

In 1993 it was reported that yeast cells that survive deletion of telomerase activ-

[1]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA and CEGMR, KAU, Jeddah, Saudi Arabia. [2]Cancer Research Unit, Children's Medical Research Institute, Westmead, NSW 2145 and Sydney Medical School, University of Sydney, NSW 2006, Australia. E-mail: jerry.shay@utsouthwestern.edu



Telomerase and ALT In cancer cells, telomeres (red) may be lengthened by telomerase, which reverse transcribes the template region of its RNA subunit (hTR), or by ALT, in which a telomeric DNA template is copied. The template may be the telomere of a nonhomologous chromosome, or it could include extrachromosomal telomeric DNA in circular (illustrated) or linear form, another region of the same telomere via loop formation, or the telomere of a sister chromatid (*11*). A combination of telomerase and ALT inhibitors is predicted to be an effective strategy for treating cancer.

ity are dependent on genes required for homologous recombination (*8*). In the following year, a small subset of human primary tumors (*2*) and immortalized cell lines (*2*, *9*) was found to be telomerase-negative. Based on the yeast cell findings that telomere length can be maintained in the absence of telomerase, it was deduced that one or more alternative, telomerase-independent mechanisms exist in human cells (*10*). ALT involves copying of a telomeric template DNA via homologous recombination (see the figure). Cells that use ALT to overcome telomere shortening have many unusual characteristics (*5*, *10*, *11*), such as highly heterogeneous telomere lengths and abundant extrachromosomal telomeric DNA. Although we are beginning to understand some of the molecular details of ALT, there has not been any progress in developing anticancer therapeutics targeting the pathway. Moreover, the possibility of more than one ALT mechanism in human cells has not yet been ruled out.

ALT occurs in a wide range of tumor types, and is a common telomere-lengthening mechanism in tumors such as osteosarcomas, undifferentiated pleomorphic sarcomas, leiomyosarcomas, astrocytic tumors grades 2 and 3, and pancreatic neuroendocrine tumors (*11*, *12*). The clinical consequences of ALT require more detailed study, but appear to be highly dependent on tumor type, with a notably worse outcome for individuals who have ALT-positive liposarcomas and a better outcome for adults with ALT-positive glioblastoma multiforme (*11*). Presumably, the prognosis is dictated by the sum total of genetic changes associated with activation of ALT in a particular tumor type, rather than the telomere-lengthening mechanism alone. Future work will require the development of simple blood tests, such as the C-circle assay (*13*), to identify ALT-expressing cancers to better determine the prognostic value of ALT in a variety of cancers.

ALT is relatively rare in the most common types of cancers, carcinomas, which are derived from epithelia. One reason could be that some highly proliferative epithelial tissues normally express regulated levels of telomerase, which partially compensates for telomere shortening to permit the cellular proliferation required over a human lifetime. Therefore, during oncogenesis these cells may be more likely to maintain their telomeres by up-regulating telomerase expression than by engaging ALT. However, these cells could still engage ALT if telomerase is inhibited by drugs during cancer therapy.

How is ALT activated in some cancer cells? It was recently observed that ALT-positive pancreatic neuroendocrine tumors and tumors of the central nervous system have mutations in or lose normal expression of the ATRX or DAXX proteins, and/or histone H3.3 (*14*, *15*). Because ATRX and DAXX act together to deposit H3.3 and remodel telomeric chromatin, it was proposed that this may block telomeric recombination and repress ALT, and that loss of function of these three proteins contributes to derepression of ALT (*14*, *15*). It will be important to determine whether these changes are sufficient for activation of ALT or whether additional changes are required.

Telomerase is expressed in the great majority of malignant tumors (*2*, *3*) and thus holds great promise as a critical and cancer cell–specific drug target. Indeed, there are several telomerase therapies in clinical trials, all of which are effective in the preclinical setting. These include the enzymatic activity inhibitor imetelstat (non–small cell lung cancer, multiple myeloma, and breast cancer, phase 2); an immunotherapy targeting telomerase epitopes expressed only on cancer cells (pancreatic cancer, phase 3); and a virotherapy that includes the hTERT promoter regulating critical adenoviral genes and thus only kills telomerase-positive cells (*4*). However, as with any targeted cancer therapy, the development of resistance is a potential threat, and malignancies that initially have only telomerase activity (i.e., the great majority) could become resistant to telomerase inhibitors by activating the ALT mechanism. In vitro experiments suggest that this is relatively uncommon, but the likelihood of resistance by this mechanism cannot be dismissed because the number of cells in typical cell culture experiments is many orders of magnitude lower than in clinically important tumors. In support of this conclusion, telomerase-positive T cell lymphomas arising in mice engineered to have telomeres of limiting length and from which telomerase activation was subsequently withdrawn entered a period of slow growth and then emerged with features of ALT (*6*). Another factor that will complicate treatment with telomerase inhibitors is that some tumors may have both telomerase and ALT activity (*5*, *11*). This is at least in part due to intratumoral heterogeneity, and whether both telomere-lengthening mechanisms are sometimes spontaneously activated within the same cells is unknown. Continued growth of ALT-positive cells would likely make these tumors rapidly resistant to telomerase inhibitors.

Published by AAAS

While there continues to be high enthusiasm for developing better telomerase inhibitors, there appears to be less interest in academia and industry for targeting ALT. This is perhaps because it is much less common, and there is limited information about activation of ALT in telomerase-positive cancer cells treated with telomerase inhibitors (*16*). As more studies focus on the molecular details of ALT and the mechanism(s) involved in its engagement, specific molecular targets and vulnerabilities may be identified that lead to the development of ALT inhibitors. These, similar to telomerase inhibitors, could be effective targeted therapies for specific cancer subtypes. Strategies combining inhibitors of both telomerase and ALT may then prove to be the most powerful and durable anticancer approach (see the figure).

References

1. C. W. Greider, E. H. Blackburn, *Cell* **43**, 405 (1985).
2. N.-W. Kim *et al*., *Science* **266**, 2011 (1994).
3. J. W. Shay, S. Bacchetti, *Eur. J. Cancer* **33**, 787 (1997).
4. M. M. Ouellette, W. E. Wright, J. W. Shay, *J. Cell. Mol. Med.* **15**, 1433 (2011).
5. T. M. Bryan, A. Englezou, L. Dalla-Pozza, M. A. Dunham, R. R. Reddel, *Nat. Med.* **3**, 1271 (1997).
6. J. Hu *et al*., *Cell* **148**, 651 (2012).
7. J. W. Shay, W. E. Wright, *Carcinogenesis* **26**, 867 (2005).
8. V. Lundblad, E. H. Blackburn, *Cell* **73**, 347 (1993).
9. J. P. Murnane, L. Sabatier, B. A. Marder, W. F. Morgan, *EMBO J.* **13**, 4953 (1994).
10. T. M. Bryan, A. Englezou, J. Gupta, S. Bacchetti, R. R. Reddel, *EMBO J.* **14**, 4240 (1995).
11. J. D. Henson, R. R. Reddel, *FEBS Lett.* **584**, 3800 (2010).
12. C. M. Heaphy *et al*., *Am. J. Pathol.* **179**, 1608 (2011).
13. J. D. Henson *et al*., *Nat. Biotechnol.* **27**, 1181 (2009).
14. C. M. Heaphy *et al*., *Science* **333**, 425 (2011).
15. J. Schwartzentruber *et al*., *Nature* **482**, 226 (2012).
16. O. E. Bechter, Y. Zou, W. Walker, W. E. Wright, J. W. Shay, *Cancer Res.* **64**, 3444 (2004).

10.1126/science.1222394

PLANETARY SCIENCE

Fragments of the Lunar Cataclysm

Meteorite samples returned from the Moon may reveal processes involved in the evolution of the early solar system.

Alan E. Rubin

The flux of extraterrestrial materials impacting the Earth is not representative of potential source bodies in the solar system. Except for the Moon and Mars, chunks of planets and moons are absent from our meteorite collections. And aside from tiny interplanetary dust particles, comets are also missing; they strike Earth at high relative velocities and do not leave identifiable remains. Although asteroidal fragments face a less harrowing journey to Earth, they have to endure launch from their parent body by hypervelocity collisions, impacts by micrometeorites in interplanetary space, and frictional stresses involved in passing through Earth's atmosphere. Major collisions on asteroids are stochastic events; for example, an asteroid-shattering impact 470 million years ago on a particular chondritic body (*1*) disgorged a clutch of material so massive that numerous fossil meteorites from that body have been found in Ordovician sedimentary rocks (*2*). The flux of material that reaches the top of Earth's atmosphere is the same as that impacting the lunar surface. Although we cannot identify intact meteoritic debris in terrestrial rocks that are billions of years old, there is a chance that such material would survive on the Moon. On page 1426 in this issue, Joy *et al*. (*3*) identify ancient projectile fragments in Apollo 16 lunar rocks that were consolidated sometime between 3.8 and 3.4 billion years ago (Ga). This time interval corresponds to the tail end of the epoch when large lunar impact basins like Imbrium were being formed (*4*) (see the figure).

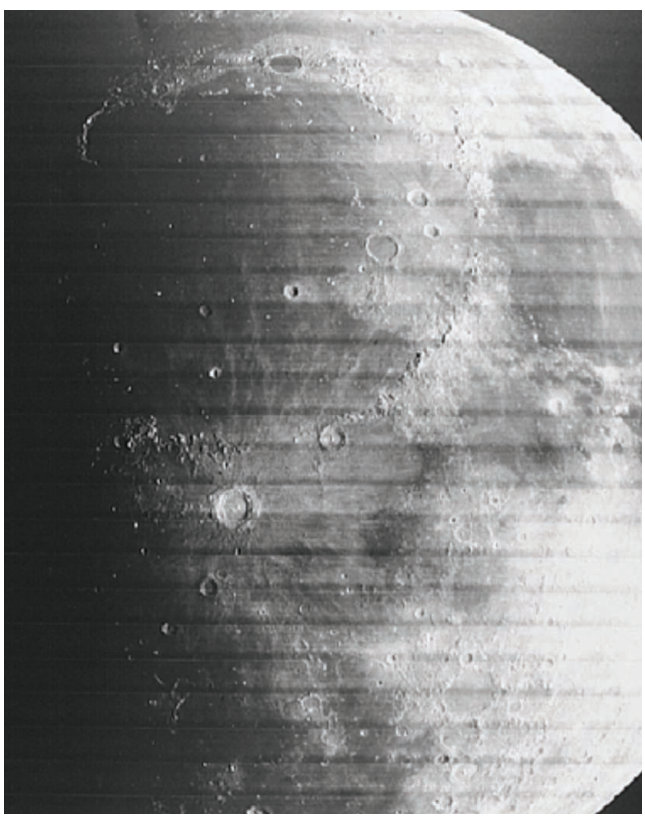


Image of an impact. The Imbrium Basin, with a diameter of 1160 km, is the largest basin on the lunar nearside. It was formed by a giant impact event ~3.85 Ga and was subsequently filled with basaltic lava. Image taken by NASA's Lunar Orbiter 4 in 1967.

In the lunar samples examined by Joy *et al*., projectile material occurs as rare ultramagnesian clasts tens to hundreds of micrometers in size. The clasts are similar in texture and mineralogy to chondrule fragments from primitive carbonaceous chondritic meteorites. The compositions of these clasts differ dramatically from those of the much-more-ferroan Moon rocks; they are also richer in Mg and poorer in Ni than terrestrial igneous rocks, indicating that they are not contaminants introduced during curation and processing.

Meteoritic materials that have been identified in younger lunar soils include carbonaceous chondrites (*5*), enstatite chondrites (*6*), mesosiderites (*7*), and irons (*8*). Although only a handful of samples are involved, their diversity roughly resembles that of modern meteorite falls on Earth. In contrast, the relative uniformity and magnesian compositions of the meteoritic material preserved in the ancient lunar regolith suggest that the flux of materials pelting the Earth and Moon ≥3.4 Ga ago was different from the modern flux.

Many researchers think that this difference was due to some catastrophic event that resulted in a shift of the abundance and types

CREDIT: NASA

Institute of Geophysics and Planetary Physics, University of California, Los Angeles, CA 90095–1567, USA. E-mail: aerubin@ucla.edu

Cancer and Telomeres—An ALTernative to Telomerase

Jerry W. Shay *et al.*

Science **336**, 1388 (2012);
DOI: 10.1126/science.1222394