Cancer's relentless evolution

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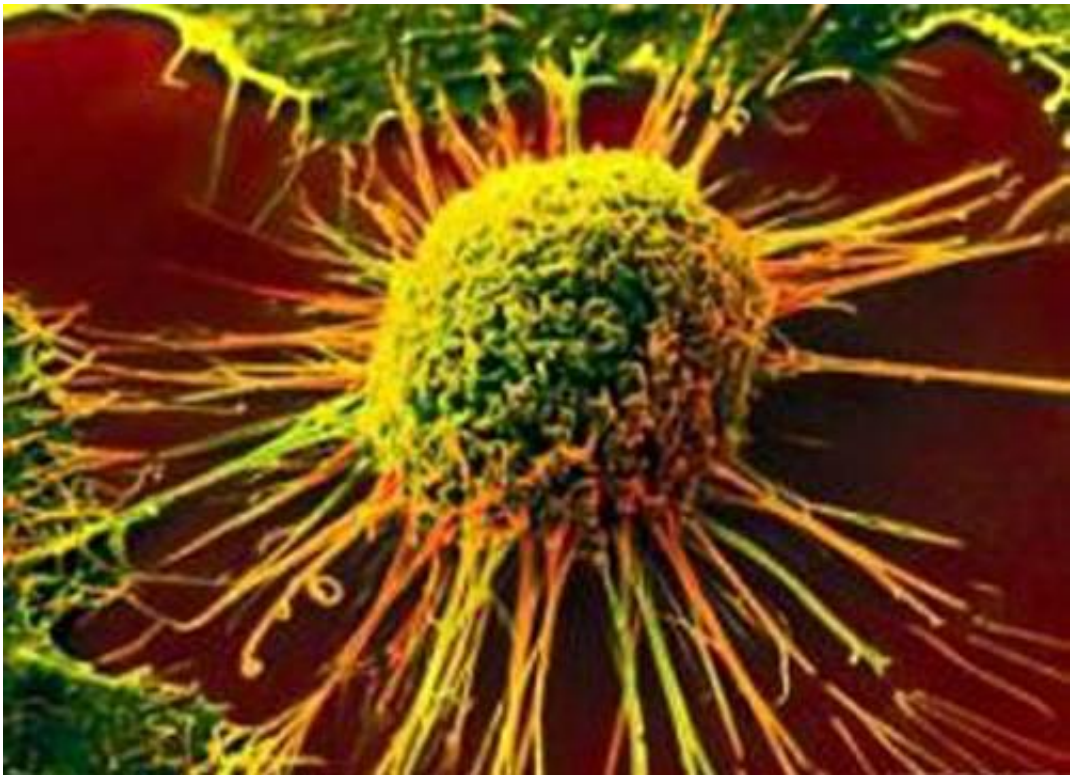

All living things—from dandelions to reindeer—evolve over time. Cancer cells are no exception, and are subject to the two overarching mechanisms described by Charles Darwin: chance mutation and natural selection.

In new research, Carlo Maley, PhD., and his colleagues describe compulsive evolution and dramatic genetic diversity in cells belonging to

one of the most treatment-resistant and lethal forms of blood cancer: acute myeloid leukemia (AML). The authors suggest the research may point to new paradigms in both the diagnosis and treatment of aggressive cancers, like AML.

Maley is a researcher at Arizona State University's Biodesign Institute and an assistant professor in ASU's School of Life Sciences. His work focuses on applying principles of evolutionary biology and ecology to the study of cancer.

The group's findings appear in this week's issue of the journal *Science Translational Medicine*.

The cells, they are a changin'

A tumor is a laboratory for evolutionary processes in which nature experiments with an immense repertoire of variants. Mutations that improve a cell's odds of survival are "selected for," while non-adaptive cells are weeded out in the evolutionary lottery.

Genetic diversity therefore provides cancer cells with a library of possibilities, with some mutations conferring heightened resistance to attack by the body's immune system and others helping malignant cells foil treatments like chemotherapy. Generally speaking, the seriousness of a given cancer diagnosis may be linked with genetic diversity in cancerous cells. High diversity means the cancer has many pathways for outsmarting treatment efforts.

The diagnosis of cancer and study of disease progression is often accomplished by examining a tumor sample containing many billions or even trillions of cells. These are subjected to so-called next generation sequencing, a technique that sifts the vast genetic composite, ferreting out sequence variants (or alleles) caused by mutations in genes. The

process then evaluates the frequency of these alleles, using the results to chart disease progression and assess the effectiveness of treatment.

According to Maley, such methods may obscure the true degree of genetic diversity, as well as the manner in which mutations arise. "One issue here is that if a mutation occurs in less than 20 percent of the cells, it's hard to detect by modern methods," he says. For example, because individual cells in the tumor probably carry unique mutations, they would be virtually impossible to observe with standard sequencing methods.

A further issue is that tracking mutations through bulk analysis of cells is typically based on certain assumptions as to how mutations arise and what their frequencies mean.

A new window

The current study attempts to provide a more accurate picture of what is taking place at the genetic level when an AML patient has a relapse or metastasis of the disease. Rather than carry out conventional bulk analysis of cells, the research group examined individual cells, screening them for the presence of two critical gene mutations common in AML, known as FLT3 and NPM1.

The results significantly alter existing assumptions of cancer progression, indicating much greater genetic diversity in AML than previously assumed. The process of convergent evolution, in which separate lineages develop similar features, appears to account for some of the observed diversity. The researchers found evidence that the exact same mutation was occuring multiple times within the same patient.

Within the paired chromosomes contained in every cell, mutations occurring on one chromosome are known as heterozygous, while those

occurring on both are homozygous. The new study shows that in AML, every possible combination of homozygous and heterozygous mutation occurs for the two gene mutations under study.

The study examined individual cells in six patients with AML. The results clearly showed all combinations of homo and heterozygous mutation. "There's no way to explain that with each mutation only happening once," Maley says. Instead, some mutations are occurring repeatedly in the same tumor. "That's scary because it means that these cancers have access to many mutations and can find the same mutation over and over."

Maley notes that influences from the environment may drive convergent evolution but that identical mutations can also arise through pure coincidence, simply by virtue of the enormous numbers involved.

A 1 cm3 AML tumor, for example, may contain a billion cells, each containing some 3 billion base pairs in its genome. Mutations are estimated to occur at a rate of 1 mutation in every billion base pairs. "That means every time the population of cells in a 1 cm tumor undergoes 1 generation, which we think takes just a couple days, every possible mutation of the genome is happening somewhere in that tumor," Maley says. This alone would lead to the same mutation likely occurring independently multiple times.

Curbing cancer's lethality

Given AML's near-limitless capacity for creating novel variants, what can clinicians do to halt the disease's pitiless advance? According to Maley, one hopeful approach would be to use cancer's evolveability to advantage, rather than attempt to fight it head on. "Can we put pressures on the tumor that select for a behavior that we want —a manageable cancer that doesn't kill us?"

This new paradigm draws on a branch of ecology known as life history theory. The idea is to carefully study the environmental factors that may lead organisms to favor either a fast reproducing or slow reproducing strategy to maximize their survivability.

Currently, most cancer therapy relies on frontal assaults on malignant cells. The approach is effective provided the given cancer is limited in the genetic variants it can produce in order to adapt to changing environments and survive. For a cancer with very high genetic diversity (like AML) however, the unintended effect of treatment is often to select for the most aggressive, resistant cells, clearing away their competitors and furnishing them with all the resources they need to flourish.

According to life history theory, fast reproduction tends to occur in environments with high extrinsic mortality. Aggressive cancer treatment creates just such an environment, favoring those cells able to reproduce quickly, producing large numbers of daughter cells, with a few evading extrinsic mortality to repopulate the tumor.

On the other hand, a very stable environment often favors slow reproduction, because organisms reach a carrying capacity of their surrounding environment. In this case, the limiting factor becomes competition between like organisms. Here, a slow reproducing strategy favoring greater investment in maintenance and survivability wins the competition.

As Maley points out, the clinical implications are clear: "This approach would say 'let's keep tumors as stable as possible and keep their resources limited.' If we are able to keep the tumor cells contained and let them fight it out, we would expect to see more competitively fit cells that are growing very slowly."

While the current single-cell analysis evaluated just two mutations in AML, the results demonstrated the staggering evoleability of this form of cancer. Eventually, researchers like Maley would like to examine whole genomes in single cells. Presently, many technical hurdles exist. Nevertheless, evolutionary approaches to cancer are already suggesting a broad rethinking of this complex of diseases.

More information: Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia, *Sci Transl Med* 1 April 2015: Vol. 7, Issue 281, p. 281re2. DOI: 10.1126/scitranslmed.aaa0763