GENOMICS

A family tree in a tumor

A new technique finds genomic subpopulations to indicate cancer progression.

When scientists discuss the vast heterogeneity of cancer cells, they are generally referring to differences between patients or perhaps between tumors. Researchers led by Michael Wigler at Cold Spring Harbor Laboratory have now found that variation *within* a tumor reveals distinct populations of cells. This could shed light on how tumors progress, a problem that is particularly difficult to study because tumor samples are generally not collected as cancer advances. In the past, tumor heterogeneity has been dismissed as a stochastic process rather than considered a clue to understanding progression, but probing for differences within tumor cells can reveal information that studying them as a single mass cannot, says lead author Nicholas Navin.

To study tumor progression, the researchers combined several techniques into a method they named sector-ploidy-profiling (SPP). First, they sectioned breast cancer tumors and isolated nuclei from the different sections. Fluorescence-activated cell sorting showed that about half the tumors were "polygenomic," containing multiple tumor subpopulations that differed in the amount of DNA in the cells. A technique to assess copy number variation (representational oligonucleotide microarray analysis) helped identify how sections of chromosomes had been broken, lost or amplified. Though both techniques are well established, says Wigler, "the idea of combining these methods to look for genomic changes within the tumor hadn't been done."

The researchers then applied a number of exploratory statistical tests to ascertain the phylogeny of subpopulations within the tumor. This showed that rather than containing cells representing a variety of intermediate states, tumors tended instead to contain large but discrete subpopulations of cells.

Many current techniques to study tumors do not account for discrete subpopulations and so could mask clinically important variations, says Navin. Although pathologists generally do examine cell appearance from several sites within the same tumor, they do not attempt to classify its diversity. Instead, they grade the tumor according to the most aggressive growth observed. Molecular analyses of tumors, which evaluate what growth factors a patient's cancer relies on, may examine cells from only one site within a tumor or mix cells from different sections. However, because tumor subpopulations can lose regions on some chromosomes while other regions become amplified, these assessments could overlook subpopulations containing either no copies or many copies of key genes, says Navin: "You wouldn't necessarily see those regions because they would get averaged out."

In a single tumor, chromosome breaks, loss and amplification can generate distinct subpopulations. D, diploid; H, hypodiploid; A, hyperaneuploid. Image courtesy of Nicholas Navin.

To understand how subpopulations might be distributed within a tumor, the researchers used fluorescence *in situ* hybridization probes within one tumor that contained several genomic subtypes. This revealed that chromosomal amplification had led to the presence of extra copies of several known oncogenes, while deletions eliminated known tumor suppressors. Other deletions and amplifications involved genes previously not associated with cancer. Furthermore, amplification within one aneuploid population had led to two distinct subpopulations that co-occupied the same sections within the tumor.

Additional work showed that two subpopulations were mixed together. Wigler sees several potential explanations: perhaps one population is invading another, or perhaps the two populations are cooperating somehow. "Maybe the cancer is more like an organ than a single population that's gone rogue," he says, adding that it's too early to know whether such speculation is justified.

Additional SPP experiments should reveal whether cancers besides breast tumors show similar patterns. Meanwhile, Navin hopes to combine SPP with single-cell analysis, allowing for a finer analysis of heterogeneity within a tumor.

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RESEARCH PAPERS
Navin, N. *et al.* Inferring tumor progression from genomic heterogeneity. *Genome Res.* published online 10 November 2009.