Discovery offers unprecedented look at regulation of gene expression

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(Medical Xpress)—A groundbreaking technique developed at the University of Virginia School of Medicine is allowing scientists to examine histone modifications of genetic loci – a process that regulates gene expression – in single cells. The researchers believe the new method will have broad applications for most biomedical areas, most immediately in atherosclerosis research.

The technique enables researchers, for the first time, to analyze the process within the individual cell types that make up complex tissues, overcoming a major limitation of traditional analysis and offering unprecedented opportunities for understanding mechanisms that contribute to development of major human diseases, including cancer, cardiovascular and neurological diseases.

The U.Va. researchers believe their new technique will have broad applications for most biomedical areas, including elucidating mechanisms that contribute to development of complex tissues and how cells that contain the same genetic material become different from one another during development. A particularly valuable feature of this new method is that it can be used to study mechanisms of gene regulation in single cells in fixed histological sections, including large archives of human autopsy specimens. Although the method was developed to look at histone modifications at single gene loci in individual cells, it can likely be adapted to look at any protein-DNA interactions.

"It's extremely important to understand how one set of genetic material

can be differently used by each of the many cell types that exist in your body – for example, what makes bone cells different from heart cells, tumor cells different than non-tumor cells," said Gary K. Owens, Robert M. Berne Professor of Cardiovascular Research and professor of molecular physiology and biological physics. "Our method is going to allow completely new approaches to addressing these fundamental questions."

The new method should greatly advance science's understanding of atherosclerosis – hardening of the arteries that contributes to heart attacks, stroke and hypertension – by allowing groundbreaking examination of the mechanisms that shape plaque development and whether a plaque might be vulnerable to rupture, the inciting event for adverse clinical events. The new technique already has indicated that numerous studies of atherosclerosis have grossly underestimated the frequency and role of smooth muscle cells within lesions, the U.Va. researchers report.

U.Va.'s new method overcomes several limitations of traditional testing using chromatin immunoprecipitation analysis, or ChIP. Because that form of analysis does not allow the examination of histone modification at the level of individual cells – often requiring a million cells or more – attempts to analyze multicellular tissues lead to murky results.

For example, an analysis of a tumor or atherosclerotic tissue produces a crude composite of the many different cell types in the sample.

"When ChIP assays are performed on a tumor biopsy sample," Owens said, "you get a mixture of signals not only from the cancer cells in that biopsy specimen, but all the normal cells. … Thus the ChIP assay has very limited usefulness for analyzing normal or pathological tissues."

U.Va.'s method, on the other hand, overcomes this problem by

combining two approaches known as in situ hybridization, or ISH, and proximity ligation assay, or PLA. The result is an unprecedented look, of exceptional clarity, at the histones at work shaping individual cells.

The new technique is described in an article that has been published online in the journal *Nature Methods*. The article will appear in a forthcoming print edition as well. It was written by research associate Delphine Gomez, graduate student Laura S. Shankman, research associate Anh T. Nguyen and Owens.

Developing the technique required Gomez to take a painstaking approach to integrating the ISH and PLA methods, with much trial and error. Yet her methodical work produced positive results remarkably quickly, considering the complexities involved.

"Between the beginning and the first positive results was six months," she recalled. She followed that initial gene with two more in only four weeks, demonstrating the widespread applicability of the method.

"I remember thinking she accomplished it much faster than I thought anybody could have," Owens said.

Provided by University of Virginia