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Scientists solve structure of gene regulator that plays key role in cancer

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Scientists at The Wistar Institute have collaborated on a major advance in understanding a gene regulator that contributes to some of the deadliest cancers in humans. The culmination of 10 years' work, their research paves the way for the development of new cancer therapies.

During much of its lifetime, the DNA that resides in each of our cells exists in an inactive form, stored inside densely knotted structures in the cell nucleus. There, the DNA is wound spool-like around proteins called histones, which bind to and restrict the DNA's activity. For a cell to activate a gene, many proteins, called transcription factors, work in concert to separate DNA from the histones. One family of transcription factors are enzymes known as HATs, or histone acetyltransferases, which transfer acetate groups onto the histones.

Wistar scientists, collaborating with researchers at the Johns Hopkins University School of Medicine, have made a major advance in the understanding of the structure and function of a key HAT enzyme called p300/CBP.

Unlike most HATs, which regulate the expression of only a few genes, p300/CBP is involved in the activation of a wide variety of genes. In addition, aberrant p300/CBP activity contributes to pancreatic, colon, and lung cancer – among the deadliest cancers in humans – as well as gastric and thyroid cancer and some leukemias. In addition to acting as an oncoprotein by promoting tumors, p300/CBP also can suppress tumors.

These unusual properties have made p300/CBP one of the most studied enzymes in the HAT family, and a target for developing new anti-cancer drugs, says Ronen Marmorstein, Ph.D., a professor in the Gene Expression and Regulation Program at Wistar and a senior author and corresponding author on the study. Philip A. Cole at Johns Hopkins is also a senior author and corresponding author.

“It’s unusual to have a HAT that’s so implicated in cancer, and even more unusual to have one that has both tumor suppressor and oncoprotein activities,” Marmorstein says.

In a report published in the Feb. 14 issue of the journal *Nature*, Marmorstein, Cole and their colleagues detail their elucidation of the three-dimensional structure of a p300/CBP HAT domain, or segment, bound to a small molecule that inhibits its activity. The study also reveals how the binding site and chemical mechanism of the enzyme enable it to regulate a wide variety of genes.

p300/CBP has long been recognized for its ability to involve other transcription factors in regulating gene expression. About 10 years ago, when scientists discovered that p300/CBP also had histone acetyltransferase activity, Marmorstein and his group began work to determine its three-dimensional structure.

But solving that structure was no easy feat. The researchers attempted to create crystals of p300/CBP to analyze using x-ray crystallography, a widely used analytical technique in which x-rays are beamed at crystals containing the protein of interest. The three-dimensional structure of the crystallized protein is deduced by analyzing the pattern of x-ray diffraction caused by the arrangement of atoms in the protein crystal.

Marmorstein and his colleagues spent years attempting to crystallize p300/CBP – a process made excruciatingly difficult by the protein’s

tendency to lose its functional form upon isolation for crystallization.

In 2004 a breakthrough was made when Paul Thompson, a postdoctoral fellow in the Cole laboratory at the time and coauthor of the current study, discovered why the protein performed so poorly: p300/CBP not only works to acetylate histones but also acetylates itself.

Thompson found a way to prevent this self-acetylation using a “chemical trick” to produce the protein in a form that contained no acetyl groups. By employing a few additional tricks to counteract the enzyme’s flexible and “floppy” nature, the scientists were then able to crystallize it.

Studies of the structure show that p300/CBP contains a binding pocket that is suitable for associating with a wide range of substrates – the molecules it binds with – and makes p300/CBP more “promiscuous” than other HATs, Marmorstein says.

In addition, p300/CBP uses a novel “hit-and-run” chemical mechanism to convert its substrates to the resulting protein products. The chemical mechanism differs from those employed by other HATs in that the histone substrate binds only transiently, leaving after a very brief encounter.

This hit-and-run mechanism is consistent with the enzyme’s ability to acetylate a variety of substrates because they don’t have to bind in a very stable fashion, Marmorstein says.

The chemical mechanism employed by p300/CBP also bodes well for designing cancer drugs capable of pinpointing p300/CBP without affecting other enzymes – and causing unwanted side effects. “Because of p300/CBP’s chemical mechanism, which differs from that of other HATs, an inhibitor that works against this family of enzymes likely won’t work against the other ones,” Marmorstein explains.

The scientists are now working to further elucidate the functions of p300/CBP and to solve larger structures of the protein. They plan to use the information they have already gained to develop inhibitors of p300/CBP activity – research that will pave the way for the development of new anti-cancer therapies, Marmorstein says.

Source: The Wistar Institute