Scientists find new structural motif in key enzymes is essential to prevent autoimmune disease

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Scientists from the Scripps Research Institute and the Genomics Institute of the Novartis Research Foundation have found a specific mutation that leads to the development of severe autoimmune kidney disease in mice. The research sheds light on the basic biology of the immune system, as well as on the effectiveness of drugs such as the anti-leukemia medication Gleevec/Imatinib.

The study was published in the January 16, 2009 edition (Volume 33, No. 1) of the journal *Molecular Cell*.

In the study, the scientists identify a disease-causing mutation in a binding structure common to dozens of kinases—specific enzymes, especially important in cell signaling, that can modify other proteins by transferring a phosphate group onto them. The mutation reduced the activity of an important kinase, Lyn (a member of the Src family, which modulates important cellular processes including cell migration, proliferation, and differentiation).

"Our study has several important implications," said Karsten Sauer, a Scripps Research scientist and assistant professor who led the study. "First, it shows that when you eliminate the activity of the Lyn kinase through mutation, you develop problems in B cell signaling, resulting in B cell hyperactivity which leads to a severe autoimmune reaction—in this case, autoimmune glomerulonephritis, a form of kidney disease very

similar to human lupus. This shows for first time how essential the Lyn kinase activity, and not potential adaptor or scaffold functions of the protein, is for B cell signaling, and for preventing autoimmune disease."

B cells produce pathogen-fighting antibodies and are a critical part of the adaptive immune system.

The study showed that, in so-called "WeeB" mice, mutational disruption of a binding structure results in expression of a Lyn protein with reduced catalytic activity and disturbed B cell receptor signaling. These mice show profound defects in B cell development and function and quickly succumb to the kidney disease.

The structure in which the mutation occurs, called a G-loop, allows for the process of adenosine triphosphate (ATP) binding—the cell's main energy source—and phosphate transfer in the protein kinases. The structure also controls the binding of ATP competitive compounds, such as Imatinib (Gleevec®), the chronic myeloid leukemia (CML) treatment that is the first approved drug targeting another kinase, Abl, in rapidly dividing cancer cells.

The WeeB mutation disrupts a molecular "bridge" within the Lyn G-loop that, Sauer and colleagues found, stabilizes the structure, limiting its inherent flexibility, and contributes to proper ATP binding and the transfer of the phosphate group during catalysis.

Multidisciplinary Approach

Sauer and his colleagues used a multidisciplinary approach to characterize the G-loop salt bridge, combining molecular dynamics with structural modeling, biochemistry, and mouse genetics to decipher the importance of this structure.

In the molecular dynamics analysis of the normal or mutant G-loop structure, for example, the scientists produced some 2.5 million simulation steps of the various possibilities of the structure's movement, using the Scripps Research supercomputer cluster. The scientists found that removing the salt bridge makes the G-loop and other regions of the Lyn kinase domain much more flexible, weakening its ATP interactions and reducing catalytic activity.

"Intriguingly, we found through molecular modeling that the G-loop salt bridge is also important for therapeutic inhibitor binding, using Abl as an example," Sauer said. "Thus, in addition to unveiling the specific roles of the Lyn kinase activity in signaling, our approach, which couldn't be much broader, offers a fundamental insight into the molecular mechanisms through which kinases function, and how they interact with ATP-competitive pharmacologic inhibitors. This could help in the development of more kinase therapeutics like Gleevec."

The search for specific protein kinase inhibitors is an intense area of research because of its enormous potential for drug development.

The results of the new study also reveal a widely diverse group of kinases that share this G-loop salt bridge structure—nearly 60 protein kinases from plants to humans. "Intriguingly, mutations that abrogate the salt bridge have been implicated in resistance to Gleevec," Sauer said. "Structural analysis of Abl shows that these mutations reduce Gleevec binding to Abl. It has also been shown that the mutations worsen prognosis of Gleevec-treated patients, suggesting that the Abl G-loop salt bridge is required for the drug's binding and efficacy in patients."

Sauer hopes that the team's multidisciplinary approach—where molecular dynamics and structural modeling result in hypotheses that instruct biochemistry, whose results in turn direct functional studies in cells and whole animals—will encourage a more intense use of similar

approaches by others.

"Current experimental methods to analyze the dynamic motions of protein structures are limited to small protein fragments or to low resolution analysis," he said. "Luckily, computational power has increased enough in recent years to make large-scale in silico [computer-based] approaches feasible, such as molecular dynamics simulations of the effects of many different mutations on the Brownian motion of the protein atoms, or as we did in the case of Lyn and Abl, on ATP or inhibitor binding to kinase domains. Modern computational technology also enabled virtual ligand screens where one simulates the binding of many different compounds to, say, an ATP binding pocket to design the most potent kinase inhibitor drugs. Of course, such computer simulations cannot replace 'wet' experiments at the bench, but they can speed up the drug discovery process significantly by focusing the researcher's experimental efforts onto those compound scaffolds that are most likely to succeed."

In the current study, computational methods enabled the scientists to formulate a hypothesis for how the salt bridge mutation in the "WeeB" mice impacts kinase domain function. The scientists then tested the hypothesis by specific experiments—site-directed mutagenesis of the other salt bridge forming residue, or removal of the salt bridge in additional kinases besides Lyn, followed by kinase assays to test the prediction that this also reduced activity.

"If I dream up a little castle in the clouds," Sauer said, "virtual technologies could possibly one day help us to outsmart cancer cells that mutate residues in the kinase ATP binding pocket or in other drug target domains to become resistant to drug treatment. The possibility that one could predict all potential drug resistance causing mutations and design compounds that will inhibit them if they arise is fascinating and holds significant therapeutic potential."

Source: Scripps Research Institute