Key site in iron metabolism aids in diagnosing anemia of chronic disease

August 5 2008

University of Utah School of Medicine researchers have developed a new tool that facilitates diagnosis of anemia related to chronic illness, as well as diseases of iron overload. The results of a study detailing the new tool are published in the August 2008 issue of the journal *Cell Metabolism,* a publication of Cell Press.

Iron balance in the body is regulated by the interaction between a liver-produced hormone called hepcidin and the iron transporting receptor ferroportin. Hepcidin binds to ferroportin resulting in decreased export of iron out of cells. An excess of hepcidin in the blood can result in anemia and a deficiency of hepcidin causes a build-up of iron that is damaging to body organs.

Since both anemia and iron overload have various causes, it is often difficult to distinguish among those causes. "It is hard to diagnose the anemia of chronic disease," said senior author Jerry Kaplan, Ph.D., University of Utah professor of pathology and assistant vice president for research at the University of Utah Health Sciences. "Having an assay for hepcidin would make it much easier and it would also help in diagnosing iron overload diseases."

Identification of the Hepcidin-Binding Domain

In the study, Kaplan and researchers from the University of Utah and University of California, Los Angeles report that they have identified the

hepcidin-binding domain (HBD), the specific site where hepcidin binds to ferroportin. By placing a synthetic version of that binding site on agarose beads, the researchers developed a rapid, sensitive test, called the HBD assay, for measuring the concentration of active hepcidin in the blood.

The ability to detect and measure hepcidin has important implications for the diagnosis of anemias and iron overload disorders related to hepcidin. Anemia is a deficiency of the oxygen-carrying molecules inside red blood cells which can be caused by iron deficiency, vitamin B12 or folate deficiency, or chronic illnesses. Anemia of chronic disease, or anemia of inflammation, is a form of anemia that is thought to be related to abnormally high levels of hepcidin.

The most common human disorder of iron overload is hereditary hemochromatosis, which leads to abnormal accumulation of iron in the liver, heart, skin, and other organs. Some types of hereditary hemochromatosis are associated with inappropriately low levels of hepcidin in the blood.

The HBD assay developed by Kaplan and his colleagues detects biologically active hepcidin. This assay can readily detect variations in hepcidin levels in the blood due to mutations in genes that are known to affect hepcidin levels, as well as mutations in other genes involved in iron metabolism. It can also measure hepcidin concentration in response to inflammation. This novel test would allow doctors to distinguish anemias and diseases of iron metabolism that arise from abnormalities in hepcidin from those that have other causes.

Hepcidin was first reported for its role in the body's defense against bacterial and fungal infections. Current scientific evidence, however, suggests that hepcidin's primary role in the body is to regulate iron balance.

Kaplan and his colleagues found that even very small changes to the composition of the HBD had significant effects on the ability of the binding site to bind hepcidin. They also discovered that hepcidin's ability to bind to the HBD decreases at temperatures below the normal human body temperature of 37°C due to structural changes in the hepcidin molecule at lower temperatures. This change in structure also affected the ability of hepcidin to bind to bacteria. This raised questions about the effect of low temperatures on iron metabolism and antibacterial activity.

Evolutionary Insight

The hepcidin-binding domain of fish is nearly identical to the human HBD. The researchers looked at hepcidin in fish such as the brown trout from the Middle Provo River, which routinely live in very cold waters. Most mammals have only one hepcidin gene, but fish have multiple hepcidin genes that encode hepcidin molecules of different lengths. In this study, Kaplan and his colleagues found that the fish hepcidin which is the same length as human hepcidin was able to bind to the HBD at temperatures as low as 4°C but had very little antibacterial activity at both 4°C and 37°C. This discovery provides insight into the evolution of hepcidin among vertebrates. Human hepcidin has both iron- and bacteria-related activities, while fish hepcidin genes evolved to separate these functions.

Due to the similarity of the hepcidin binding site among vertebrates, the usefulness of the novel HBD assay described in this study is not limited to humans. "The assay can be used to easily measure hepcidin in the blood of all vertebrates," says Kaplan.

Source: University of Utah