Major 'missed' biochemical pathway emerges as important in virtually all cells

A new study by Duke University researchers provides more evidence that the nitric oxide (NO) system in the life of a cell plays a key role in disease, and the findings point to ways to improve treatment of illnesses such as heart disease and cancer.

The new findings, which Stamler said change understanding of how the nitric oxide system is controlled, appear in the May 23 issue of the journal *Science*.

The nitric oxide system in cells is "a major biological signaling pathway that has been missed with regard to the way it controls proteins," and it is linked to cancer and other diseases when the system goes awry, said Jonathan Stamler, M.D., a professor of medicine and biochemistry at Duke University Medical Center who worked on the study.

In the body, nitric oxide plays a role in the transport of oxygen to tissues and physiological activities such as the transmission of nerve impulses, and the beating of the heart. When things go awry with the nitric oxide system, bad things can happen in bodies, according to recent studies. For instance, there may be too little nitric oxide in atherosclerosis and there may be too much in Parkinson's disease; there may not be enough nitric oxide in sickle cell disease and there may be too much in some types of diabetes, Stamler said.

"What we see now for the first time in the *Science* paper is that there are enzymes that are removing NO from proteins to control protein activity," Stamler said. "This action has a broad-based effect, frankly, and probably happens in virtually all cells and across all protein classes. Nitric oxide is implicated in many disease processes. Sepsis, asthma, cystic fibrosis, Parkinson's disease, heart failure, malignant hyperthermia -- all of these diseases are linked to aberrant nitric-oxide-based signaling."

An important factor that previously wasn't appreciated, he said, is that the target of nitric oxide in disease is different in every case. The finding of how nitric oxide binding to proteins is regulated opens the field for new refinement in biochemical research, said Stamler, who has been studying nitric oxide in cells for 15 years.

"Now we will need to study whether the aberrant cell signals are a matter of too much NO being produced and added to proteins or not enough being removed from proteins," he said. "It is not simply a matter of too much or too little NO being in cells, but rather how much is being added or taken away from specific proteins, which is quite a different thing."

First author on the paper, Moran Benhar, Ph.D., and co-author Douglas Hess, Ph.D., are both in the Duke Department of Medicine. Co-author Michael Forrester is a graduate student in the Duke Department of Biochemistry.

The research explains that the enzymes thioredoxin 1 and thioredoxin 2 remove nitric oxide from the amino acid cysteine within mammalian cells, thereby regulating several different actions in cells. One result of this removal is the activation of molecules that begin apoptosis, which is the

normal programmed death of a cell. This process has potential importance for many diseases, including inflammatory diseases, heart failure and cancer. Because thioredoxins are established targets of drug therapy for arthritis, the research suggests potential therapeutic applications of the process.

The nitric oxide system is analogous to the much more studied phosphorylation system, in which phosphates are added and removed from proteins, the paper said. Changes in phosphorylation are among the most common causes of disease, and proteins that regulate phosphorylation are major drug targets, Stamler said.

"Aberrant dephosphosphorylation causes disease. Expect the same for denitrosylation," Stamler said.

Similar research at Duke that was published in the journal Nature on March 16 supports Stamler's findings. Christopher Counter, an associate professor in the Duke Department of Pharmacology and Cancer Biology, and colleagues found that eNOS (endothelial nitric oxide synthase), an enzyme that enhances the creation of nitric oxide, promoted tumor development and tumor maintenance in mice.

"The Chris Counter work is especially exciting because he shows that a nitric oxide synthase is linked to cancer, and he specifically identifies the protein that is the target of the nitric oxide, the protein that gets turned on through S-nitrosylation," Stamler said. Blocking S-nitrosylation of this protein prevented cancer.

The steady stream of new papers on nitric oxide seems to underscore Stamler's long-held belief that nitric oxide affects cells in bigger ways than many had appreciated. "When we began our studies two decades ago, we hypothesized that nitric oxide was part of a significant, broad-based system," Stamler said. "Our hypothesis never changed."