From Menace to Marvel:

The Story of Nitric Oxide

By Lucie Guo

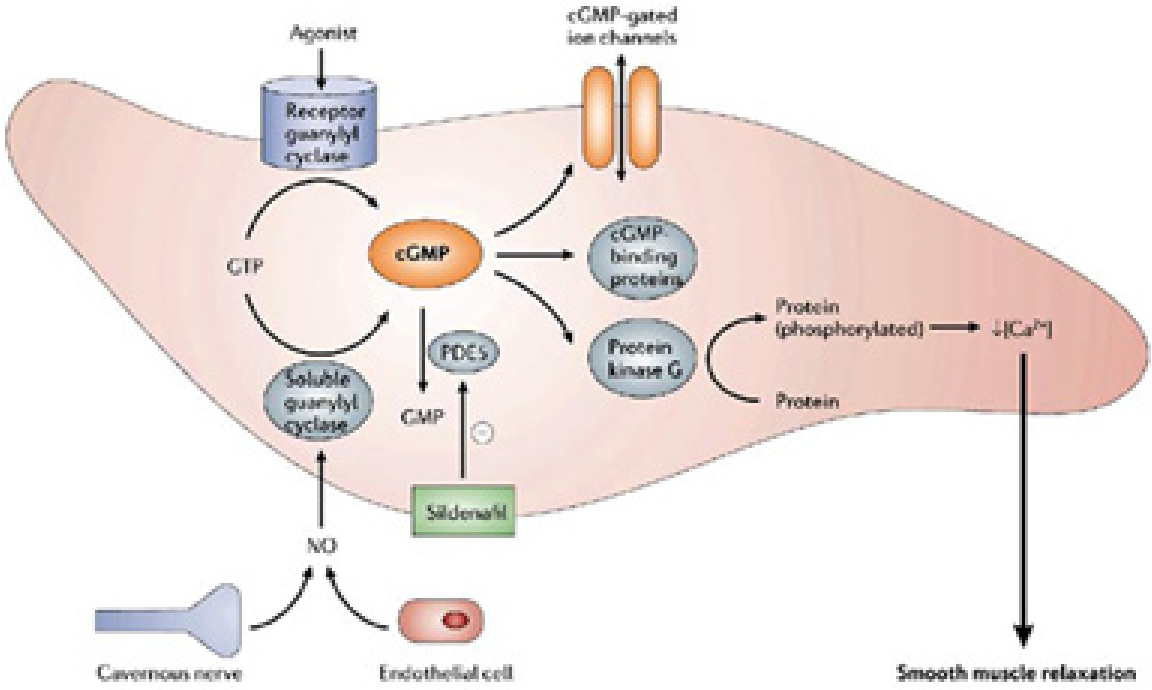
"The discovery of nitric oxide (NO) – a seemingly simple and short-lived gas molecule – as a key signaling molecule in the cardiovascular system was a key scientific breakthrough of the past decade."

In 1863, Alfred Nobel invented dynamite, the revolutionary explosive of which nitroglycerin is the key component. When he suffered from chest pain in later life, his doctor prescribed nitroglycerin. This prompted Nobel to write in a letter, "It is ironical that I am now ordered by my physician to eat nitroglycerin." More than 100 years later, it was discovered that nitroglycerin worked by releasing nitric oxide. The discovery of nitric oxide (NO) – a seemingly simple and short-lived gas molecule – as a key signaling molecule in the cardiovascular system was a key scientific breakthrough of the past decade. The discovery that a gas molecule can transmit signals generated from one cell to another by directly penetrating through the membranes of another cell to regulate its function shocked the world and gave birth to a completely new principle for biological signaling. Fittingly, the discovery was awarded the 1998 Nobel Prize in Medicine.

Since then, researchers continue to flood journals with new insights into the biological roles and potential clinical uses of nitric oxide. An enormous effort has been made by scientists to unravel the wide array of functions of nitric oxide from its basic chemistry to its role in physiological processes. From the 1980s to today, nitric oxide has been shown to be important in an assortment of different processes, including blood pressure regulation, cancer progression, inflammation, neurotransmission, liver cirrhosis, wound repair, and diabetes. However, NO is a complex molecule that often appears to play contradictory roles. For example, the gas molecule has been reported to both promote the progression of cancer and serve as a tumoricidal agent. This review aims to trace the history by which the importance of nitric oxide was realized, as researchers over the past decade have attempted to answer the question: what is nitric oxide really doing in the body?

NO and Heart Disease

Before its discovery as an important signaling molecule in the body, nitric oxide was known as an environmental pollutant. The burning of fossil fuels emits nitric oxide, which reacts readily with oxygen, producing smog and creating nitric acid, which contributes to acid rain and depletion of the ozone. In the body, nitric oxide is made from arginine by the action of nitric oxide synthase and has a half-life of only a couple of seconds (1). Because it



LEFT: The role of NO in mediating relaxation of smooth muscle, penile erection, and pulmonary vasodilatation.

is soluble in both aqueous and lipid, polar and nonpolar environments, and because of its versatility as a small gas molecule, NO can easily diffuse through the cytoplasm and plasma membrane of living cells.

Nitric oxide produced in the epithelium of blood vessels can increase blood flow and reduce the narrowing of arteries. Robert Furchgott, a New-York based pharmacologist was the first to study the relaxation of arterial smooth muscle by a compound known as acetylcholine. His research showed that the action of acetylcholine stimulated the endothelial cells to release an unknown factor that caused relaxation of vascular smooth muscles (2). Furchgott called this unknown substance EDRF, short for endothelium-derived relaxing factor.

It was not until six years later that the mystery of the identity and chemical nature of EDRF was finally solved. Louis Ignarro, a pharmacologist in Los Angeles, discovered that EDRF was identical to nitric oxide through a series of experiments demonstrating the identical effects they had on vascular muscle (3). Another study followed shortly after, which demonstrated that nitric oxide released from endothelial cells is identical to that of EDRF in their biological activity and response to inhibitors (4). Furchgott and Ignarro – together with another American scientist named Ferid Murad – shared the Nobel Prize in Medicine in 1998.

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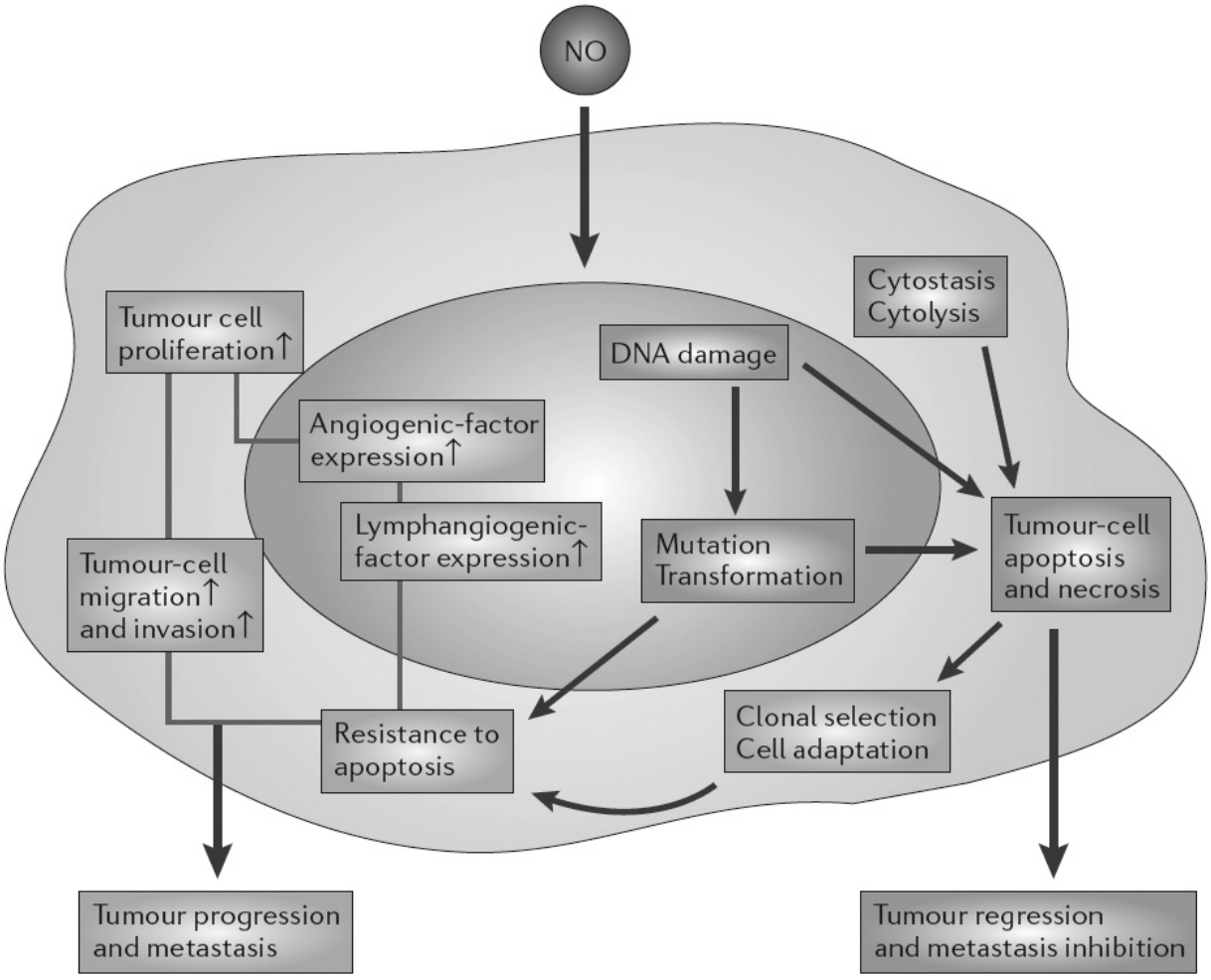
NO and Impotency

The key role nitric oxide plays in smooth muscle relaxation also makes the molecule a key target for drugs that serve to promote vasodilation in the male sexual response. Nitric oxide is able to bind to receptors of the enzyme guanylate cylase, causing an increase in the levels of a compound called cyclic guanosine monophosphate (cGMP), which leads to the inhibition of calcium influx into smooth muscle cells, thereby decreasing smooth muscle tension development. This function has led to the development of many nitric oxide-based drugs such as sildenafil citrate (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®), the most widely used therapeutics to treat erectile dysfunction.

NO and Sepsis

As a key vasodilator, nitric oxide also contributes to the cardiovascular features of sepsis, which is an im-



◀ LEFT: NO plays a dual role in the cell by promoting both tumor progression (green boxes) and tumor inhibition (brown boxes) pathways.

munological response to the presence of pathogens through the activation of a cascade of mediators. When this response is not well-controlled, septic shock occurs, leading to organ failure and death. Elevated production of nitric oxide is a key feature of sepsis, inducing widespread vasodilation and abnormally low blood pressure. Rates of mortality for sepsis can be as high as 90%, and certain studies rank sepsis as the 13th leading cause of death in the nation (6). Recent research on sepsis has been focused on developing therapeutics that can balance nitric oxide production by using specific inhibitors of nitric oxide synthase (7).

NO and Cancer

The role of NO in cancer progression is complex and remains somewhat poorly understood. The molecule often exerts multiple effects, and reports about its functions in carcinogenesis and metastasis are often inconsistent. Nitric oxide is produced by the nitric oxide synthase (NOS) genes, and iNOS (inducible nitric oxide synthase) is the isoform most directly involved in cancer processes. Studies done on the role of nitric oxide in cancer show that nitric oxide has a pleiotropic nature, manifesting its effects in a variety of different ways. Nitric oxide can both promote and inhibit tumor progression and metastasis, depending on the length of treatment time as well as sensitivity of cells (8).

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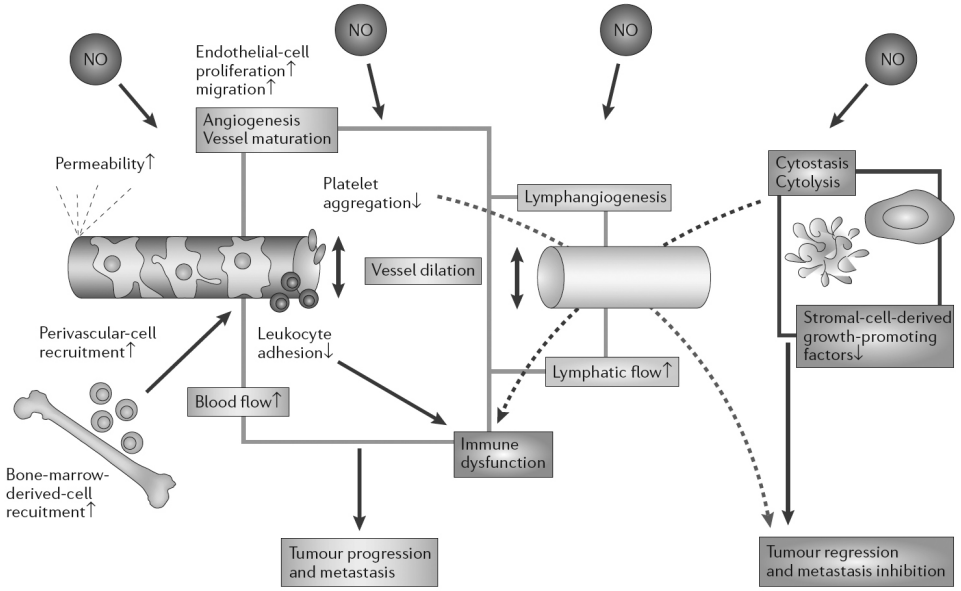
Within the tumor microenvironment, nitric oxide can be produced by cancer cells as well as by macrophages in the host. Macrophage cells in the host secrete nitric oxide as a way of exerting cytotoxic effects on cancer cells. Several pre-clinical studies have attempted to use the tumoricidal effects of nitric oxide as a strategy for treatment. It was shown that the volume of thyroid tumors can be reduced by 35% in mice injected with a plasmid vector that expresses the iNOS gene (9). Other studies have introduced cytokines that induce nitric oxide expression, including interferon-γ. Administering liposomes containing interferon-γ into metastatic cancer cells in the liver of mice resulted in an increase in nitric oxide-dependent apoptosis of tumor cells, followed by tumor regression (10).

However, even though nitric oxide



◀ LEFT: The role of NO in angiogenesis is one way the molecule induces tumor progression and metastasis.

credit: Image courtesy of Nature Publishing Group.

produced by macrophages exerts tumoricidal effects, the ability of NO to cause vasodilation makes it a facilitator in angiogenesis, the formation of new blood vessels that aid tumor development. Nitric oxide can mediate blood flow and supply of nutrients to tumors, thus promoting cancer progression. Therefore, some inhibitors of nitric oxide are being investigated as potential anti-angiogenic agents (11). iNOS expression is positively correlated with tumor progression in human gastric carcinoma and primary breast cancer (13) while promoting lymph node metastasis(12). Nitric oxide has also been reported to upregulate the expression of osteopontin, a phosphoprotein secreted by malignant tumor cells that contributes to cancer metastasis in the liver (14).

Investigations into the role of nitric oxide in cancer have revealed the complex role that the molecule plays in the disease. It is no doubt that NO is an important molecule in tumor progression, but it has been reported to play opposing roles in different environments. The elucidation of the precise role of nitric oxide necessitates further research.

The Future

The discovery of nitric oxide as a signaling molecule in the body was one of the landmark scientific findings of the past decade. It is a key player in multiple physiological processes, but often has ambivalent effects that continue to astound researchers today. Undoubtedly, nitric oxide presents itself as a key target for future drug development in a vast array of human diseases including cancer, cardiovascular disease, impotence, and septic shock. However the ambiguous effects of NO in many disease make the development of NO-based therapeutics difficult. Nonetheless, many drugs based on nitric oxide research – especially those that treat erectile dysfunction – are already widely used. The manipulation of nitric oxide production may have many therapeutic applications, but these effects may also render potential therapeutics too unspecific or perhaps even dangerous. New findings in the next decade will further establish the potential role of nitric oxide in drug development, and a precise assessment of the biological chemistry of nitric oxide may provide new understandings of how to prevent and treat many human diseases. H

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