Nitric Oxide Synthase (NOS2): A Potential Biomarker in the Colorectal Carcinogenesis

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Abstract: Nitric Oxide (NO) may also play a role in the enhancement of colon cancer risk. Inducible nitric oxide synthase (iNOS) has important functions in innate immunity and regulation of immune functions. The enzyme nitric oxide synthase (NOS) catalyzes the formation of nitric oxide (NO) and L-citrulline by the reduction of L-arginine. It has antitumor activities as well as protumor properties with various timing, concentration and tissue type. Low concentrations of NO can stimulate cell growth and protect many cell types from apoptosis, whereas high concentrations of NO can inhibit cell growth and induce apoptosis. The activation of iNOS is able to produce NO in high, potentially cytotoxic concentrations. Its effect may depend on the timing, concentration and tissue type. Low concentrations of NO can stimulate cell growth and protect many cell types from apoptosis, whereas high concentrations of NO can inhibit cell growth and induce apoptosis.

Keywords: Colo-rectal cancer, iNOS, Nitric Oxide, Biomarker

Introduction:
Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality. Main risk factors include advanced age, family history, male sex, colon polyps, and long-standing ulcerative colitis and lifestyle factors. Screening can reduce incidence and death from colorectal cancer (Grisham, 1994; Kolligs, 2016). Colorectal cancer taken together constitutes one of the most common cancers worldwide with a broad range of etiological mechanisms. Colorectal cancer is a malignant tumor arising from the inner wall of the large intestine (Seril et al., 2003). Most colorectal cancers develop from polyps and it may have no symptoms with formation of tumor. Tumors are categorized into two viz. benign tumors and malignant (cancerous) tumors. The benign tumor grows only in the tissue from which it arises and localizes in the particular area whereas benign tumors can grow quite large or rapidly with severe symptoms, and ultimate death (Hao et al., 2001). Benign tumor is unable to shed cells into the blood and lymphatic system and unable to travel to various places in the body and grow whereas others can shed cells, float like dandelion seeds in the wind through the blood or lymphatic system, land in tissues distant from the primary tumor and grow into new tumors in these distant tissues. This process of spreading to distant tissues is called metastasis (Hao et al., 2001; Bing et al., 2001).

Nitric oxide (NO) is a short-lived pleiotropic regulator and is required for numerous pathophysiological functions, including macrophage-mediated immunity and cancer (Thomas et al., 2008; Liu and Feng, 2012). Nitric oxide synthases (NOS) are expressed in colorectal cancer. NO is also shown to either promote or inhibit tumor growth of colorectal cancer cells (Bogdan, 2001; Janakiram and Rao, 2012) with concentration-dependent manner. Chronic inflammatory conditions leads to the formation of reactive intermediates of NO that is initiated by inducible nitric oxide synthase (iNOS) with the degree of tumor angiogenesis in human colorectal cancer (MacMicking et al., 1997; Chakravortty and Hensel, 2003). The tumor angiogenesis are mutagenic with acceleration of DNA damage or impairment of DNA repair, alteration of cell signalling and contribution of carcinogenesis (Bogdan, 2001; Zafirellis et al., 2010). The aim of the review is to correlate the expression of iNOS with colorectal cancer.

Colo-rectal Cancer: A Major Health Problem:

Colo-Rectal Cancer (CRC) is the most common cause of deaths from cancer that is not directly attributable to tobacco usage in some of these countries (Roberts et al., 2001). A high incidence of CRC is observed consistently in populations with a “Western-type” diet (highly caloric food rich in animal fat) combined with a sedentary lifestyle (Gochman et al., 2012). The colon and the rectum are the final portions of the tube that extends from the mouth to the anus. The food that is not digested and absorbed enters the large intestine or colon and finally the rectum. The large intestine acts primarily as a storage facility for waste; however, additional water, salts, and
Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in India. CRC appearing on the ground of inflammatory bowel disease is the result of a process which is believed to begin from no dysplasia progressing to indefinite dysplasia, low-grade dysplasia, high-grade dysplasia and finally to invasive adenocarcinoma, although colorectal cancer can arise without proceeding through each of these steps (Kolligs, 2016). Colorectal cancers most commonly spread first to local lymph nodes before traveling to distant organs. Once local lymph nodes are involved, spread to the liver, the abdominal cavity, and the lung are the next most common destinations of metastatic spread. It starts when the process of the normal replacement of lining cells goes away. Abnormal cells grow and divide and lead to growths within the colon called polyps (Seril et al., 2003; Kolligs, 2016). Polyps vary in type, but many are precancerous tumors that grow slowly over the course of years and do not spread. As polyps grow, additional genetic mutations further destabilize the cells and can make the cells more bizarre (Seril et al., 2003). When these precancerous tumors change direction and invade other layers of the submucosa or muscular layer of large intestine, the precancerous polyp has become cancerous.

Once a colorectal cancer forms, it begins to grow in two ways. Firstly the cancer can grow locally and extend through the wall of the intestine and invade adjacent structures with production of mass called primary tumor and it is more problematic and harder to remove. Local extension causes additional symptoms such as pain or fullness, perforation of the colon, or blockages of the colon or nearby structures (Seril et al., 2003; Kolligs, 2016) Secondly, as the cancer grows it begins the process of metastasis, shedding thousands of cells a day into the blood and lymphatic system that can cause cancers to form in distant locations. Metastasis, and therefore the use of the term “carcinoma” for tumours of the colon and rectum, requires invasion through the muscularis mucosae into the submucosa (Bing et al., 2001). More than 90% of colorectal carcinomas (CRC) are adenocarcinomas. Chronic inflammatory bowel diseases are also etiological factors for CRC such as ulcerative colitis, Crohn disease, and Schistosoma mansoni infection (Ekbohm et al., 1991). Tumour stage in anatomical manner, is the strongest prognostic factor for CRC. Other prognostic factors include the morphology, lymph node metastases, extend of resection, extramural venous invasion and genes and biomarkers (Kolligs, 2016).

Inflammation and iNOS

Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine (Chan et al., 2001). NO, an important cellular signaling molecule helps to modulate vascular tone, insulin secretion, airway tone, and peristalsis, and is involved in angiogenesis and neural development (Espey et al., 2000). Although synthesis of high amounts of nitric oxide (NO) by iNOS has been demonstrated in pathophysiological processes, such as acute or chronic inflammation and tumorigenesis, it may function as a retrograde neurotransmitter. NO is synthesized by a family of three NO synthase (NOS) isoenzymes that convert L-arginine into L-citrulline in the presence of molecular oxygen yielding free NO (Forstermann and Kleinert 1995). Nitric oxide is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS) (Bing et al., 2001). Inflammation is driven by the accumulation of various immune and inflammatory cells and soluble inflammatory mediators, such as cytokines, chemokines, growth factors, lipid molecules, reactive oxygen, and nitrogen species (Roberts et al., 2001). A key component of inflammation promoting cancer is the transcription factor NF-kB, which is known to play a critical role in the regulation of the inducible nitric oxide synthase (iNOS) gene. The role of nitric oxide (NO) in colon cancer remains controversial. Inducible nitric oxide synthase (iNOS) has been reported to be up regulated and down regulated in colorectal cancer in both animal models and patient tissue samples. (Liu et al., 2003). Some clinical studies have shown that iNOS increased significantly in colon adenoma and carcinoma with little or no expression in normal colon tissue (Yagihashi et al., 2003), whereas other studies report iNOS expression is decreased in colon cancer compared with high expression in normal colon tissue (Kojima et al., 1999). This free diatomic radical is produced when nitric oxide synthases (NOSs) catalyze the conversion of $^{14}N$-arginine into nitric oxide (NO) in the presence of molecular oxygen (Forstermann and Kleinert 1995).
arginine to L-citrulline. There are three isoforms of NOS i.e. NOS1, NOS2 and NOS3 those are classified following the order of cloning of the respective genes. Analysis of human biopsies of colitis and colon cancer using immunohistochemistry revealed elevated iNOS protein expression levels, which were strongly paralleled by increased expression of nitrotyrosine suggesting that iNOS has been highly activated in these tissues (Gochman et al., 2012). Two of them, endothelial NOS and neuronal NOS, are calcium-dependent, constitutively expressed and responsible for low levels of NO production (pico molar to nano molar) for short periods (minutes). The third one, inducible NOS (iNOS), is calcium-independent, not expressed in most tissues under normal conditions, but can be induced by lipopolysaccharide and various cytokines (Liu et al., 2003). NOS2 has also been termed iNOS, with ‘i’ standing both for inducible and independent (of elevated intracellular Ca2+) (Witthoft et al., 1998). This enzyme has received special attention because of its role in infection, inflammation and immune regulation.

Role of iNOS in Immune Functions

Nitric oxide (NO) is a bioactive agent that mediates a number of actions, such as vasodilatation, neurotransmission, and immune response (Knowles and Moncada, 1994). The inducible isoform, iNOS, is involved in immune response, binds calmodulin at physiologically relevant concentrations, and produces NO as an immune defense mechanism, as NO is a free radical with an unpaired electron (Kolligs, 2016). These properties may define the roles of iNOS in host immunity, enabling its participation in antimicrobial and anti-tumor activities as part of the oxidative burst of macrophages (Michel et al., 1996). It is involved in the pathogenesis and control of infectious diseases, tumors, autoimmune processes and chronic degenerative diseases. Induction of the high-output iNOS usually occurs in an oxidative environment, and thus high levels of NO have the opportunity to react with superoxide leading to peroxynitrite formation and cell toxicity (Wink et al., 1998). Because of its variety of reaction partners viz. DNA, proteins, low–molecular weight thiols, prosthetic groups, reactive oxygen intermediates, its widespread production (by three different NO synthases (NOS) and the fact that its activity is strongly influenced by its concentration (Seril et al., 2003). Analysis of human biopsies of colitis and colon cancer using immunohistochemistry revealed elevated iNOS protein expression levels, which were strongly paralleled by increased expression of nitrotyrosine suggesting that iNOS has been highly activated in these tissues (Gochman et al., 2012). The gene coding for iNOS is located on Chromosome 17 and evidence for ‘baseline’ iNOS expression has been elusive, IRF1 and NF-kB-dependent activation of the inducible NOS promoter supports an inflammation mediated stimulation of this transcript. iNOS produces large quantities of NO upon stimulation, such as by proinflammatory cytokines viz. Interleukin-1, Tumor necrosis factor alpha and Interferon gamma (Hao et al., 2001; Delker et al., 2010). Therefore, iNOS provides unique flexibility in dealing with an immune challenge. An additional level of immune regulation by iNOS is provided by the ability of the NOS enzyme to make products other than NO. These include NOHA and O2-. By generating NOHA, an inhibitor of AG activity, the iNOS system can affect pathways that mediate cell growth (ornithine to polyamines) or tissue matrices (ornithine to proline) (Bing et al., 2001). This diversity of NOS activities can produce different temporal and concentration profiles of NO as well as other products to facilitate and broaden the functional versatility of these enzymes during immune challenge.

Conclusion:

Epidemiological and clinical literature strongly implicates chronic inflammation in neoplastic diseases, especially in CRC. In inflammation and colon cancer in humans, iNOS expression and tyrosine nitration may be an indicator of cancer development and progression. Levels of NO and the site of its production by iNOS contribute to tissue damage and inflammation and are evidently important in colon cancer. It is now clear that iNOS is detrimental in inflammatory disease processes and that it helps to counteract immune reactions toward tumor cells, protects tumor cells against host immunity, and functions as an intra- and intercellular signaling molecule, shaping immune responses protective to the tumor.

Conflict of Interest:

There is no conflict of interest.

Reference:

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