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Nitric Oxide Regulates Growth Factor Signaling in Pancreatic Cancer Cells

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1. Introduction

Growth factor signaling plays a critical role in cancer proliferation and invasion. Therefore, molecules involved in growth factor signaling have become the targets of cancer therapy, and many drugs targeting growth factor signaling pathways have been developed. Some of these drugs have been used clinically, while many more are being tested in clinical trials. However, to date, molecule-targeted therapies for pancreatic cancer have not been developed.

Nitric oxide (NO) was discovered two decades ago and was initially identified as an endothelial relaxing factor. Subsequently, NO has been shown to play key roles in the post-translational modification of proteins and the regulation of protein enzymatic activity. In this paper, we present evidence indicating that NO influences cancer proliferation and invasion, and discuss the mechanisms through which NO is thought to exert these effects.

2. Production of NO in cells and tissues

NO is produced by three distinct genes products: neuronal and endothelial nitric-oxide synthases (nNOS and eNOS) and inducible nitric-oxide synthases (iNOS) (Palmer et al., 1987). The activities of nNOS and eNOS are tightly regulated by calcium-dependent calmodulin binding, whereas iNOS does not require calcium ion or posttranslational modification for its activity. As a result, iNOS expression is associated with prolonged, exaggerated NO generation of up to > 1,000-fold greater than that generated by nNOS and eNOS. Although iNOS expression is increased in macrophages and endothelial cells by various stimuli, including acute inflammation, recent studies have revealed that iNOS is expressed even in normal conditions in many tissues, including skeletal muscle and cancer (Perreault and Marette, 2001; Xie and Fidler, 1998). The expression of iNOS protein has been reported in pancreatic cancer cells, colon cancer cells, gastric cancer, breast cancer, hepatocellular carcinoma, glioma cells, melanoma cells, and laryngeal squamous cell carcinoma.
3. NO-donors

Several types of reagents called NO-donors, are capable of releasing NO constitutively (Table 1). S-Nitrosothiols (RSNO), which break down to form NO and the corresponding disulphide (RSSR), are an important class of NO-donor drugs. NO-donors containing R-NO (S-NO), are unstable and release NO upon breakdown. Decomposition of these compounds is catalysed by Cu\(^{2+}\) ions, which themselves can be formed by reduction of Cu\(^{2+}\) ions by thiols. Breakdown is accelerated by light at ultraviolet and optical wavelength and is influenced by PH.

Organic nitrates such as nitroglycerin, isosorbide dinitrate and mononitrate, which have long been used as vasodilators for the treatment of angina pectoris, release NO via both enzymatic and non-enzymatic pathways. Iron-nitrosyl complexes such as sodium nitroprusside (SNP), sydnonimine, and amine NONOate, all demonstrate NO donating capacity. NO-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs), which were developed recently, are potential anti-cancer drugs (Gao et al., 2005). NO-NSAIDs consist of a conventional NSAID to which an NO-releasing moiety is attached covalently. Glutathione S-transferase-activated NO-donors such as JS-K, have shown some therapeutic promise in cancer without hypotension (Weiss et al.).

NO-donor reagents offer a convenient source of NO for \textit{in vitro} and \textit{in vivo} experiments. Researchers can thereby avoid use NO gas but must consider intrinsic half-life, metabolites, and other activities derived from the unique moiety in choosing a NO-donor reagent.

| S-Nitrosothiols       |                                      |
|-----------------------|--------------------------------------|
| S-nitrosoglutathione (GSNO) |                                    |
| S-nitroso-N-acetylpenicillamine (SNAP) |                              |

| Organ nitrates       |                                      |
|----------------------|--------------------------------------|
| Nitroglycerin (NTG)  |                                      |
| isosorbide dinitrate (ISDN) |                                |

| Iron-nitrosyl complex |                                      |
|-----------------------|--------------------------------------|
| sodium nitroprusside (SNP) |                                     |

| Sydnonimine           |                                      |
|-----------------------|--------------------------------------|
| 3-morpholino-sydnonimine (SIN-1) |                             |
| Molsidomine           |                                      |

| Diazениндиолат (NONOate) |                                      |
|--------------------------|--------------------------------------|
| Angeli’s salt            |                                      |
| Diethylamine             |                                      |
| O2-(2,4-Dinitrophenyl)   |                                      |
| 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (JS-K) |       |

| NO-donating NSAIDs       |                                      |
|--------------------------|--------------------------------------|
| Nitric oxide-donating aspirin (NO-ASA) |                               |
| NO-naproxen              |                                      |
| NONO-ASA                 |                                      |

Table 1. NO donors
4. Actions of NO in vivo

4.1 cGMP-dependent actions

Guanylyl cyclases (GC) are expressed in the cytoplasm of almost all mammalian cells and mediate a wide range of important physiological functions, including inhibition of platelet aggregation, relaxation of smooth muscle, vasodilation, neuronal signal transduction, and immunomodulation (Collier and Vallance, 1989). GCs have evolved to synthesize cGMP in response to diverse signals, such as NO. NO activates GC by binding directly to heme to form a ferrous-nitrosyl-heme complex. Endogenous and exogenous compounds, including autacoids, hormones, neurotransmitters, and toxins, produce cellular responses through cGMP. The specificity of cellular responses to cGMP is dictated by cGMP-binding motifs in target proteins PKA (cAMP-dependent protein kinase) and PKG (cGMP-dependent protein kinase) (Francis and Corbin, 1999), cyclic nucleotide-gated cation channels (Biel et al., 1999; Kaupp, 1995) and cGMP-regulated phosphodiesterases (Beavo, 1995).

4.2 cGMP-independent actions

The major cGMP-independent actions of NO are nitrosative post-translational modifications, including protein S-nitrosylation and tyrosine nitration. Post-translational modification of proteins by S-nitrosylation, attachment of nitrosonium ion (NO) to cysteine sulphydryls, is a major mode of signaling in mammalian cells.

Indeed, critical signaling molecules and transcription factors are primary targets of NO (Stamler et al., 2001). To date, over 100 proteins have been shown to be S-nitrosylated both in vitro and in vivo. In many of these proteins, S-nitrosylation leads to functional alterations. Signaling proteins that are directly modified by S-nitrosylation include Ras, Akt, JNK, PTEN, IkB kinase, and Bcl2. (Azad et al.; Lander et al., 1997; Numajiri et al.; Park et al., 2000; Reynaert et al., 2004; Yasukawa et al., 2005)

5. Roles for NO in cancer

Conflicting results have been reported regarding the roles of NO in cancer. Recent papers reported that endogenous NO promotes oncogenesis and angiogenesis in various cancers (Ambs et al., 1998; Camp et al., 2006). In contrast, other studies have shown that NO inhibits cell proliferation and induces apoptosis in various cells including cancer cells, in vitro and in vivo (Chawla-Sarkar et al., 2003; Jarry et al., 2004; Kalivendi et al., 2001; Kotamraju et al., 2007; Notas et al., 2006; Peshes-Yaloz et al., 2007; Wang et al., 2003). These studies suggest that NO can act either as a tumor suppressor or a tumor enhancer depending on cell type and the level of NO in the cells. However, the molecular mechanism underlying the inhibitory effects of NO on cancer viability, remains unclear.

5.1 Roles in carcinogenesis and cancer promotion

NO and reactive nitrogen species (RNOS) induce the formation of nitrosamines, which can cause cancers in a wide variety of animal species. Nitrosation of nucleic acid bases leads to deamination which in turn results in mutagenic or carcinogenic conversion cytosine to uracil, guanine to xanthine, methylcytosine to thymine and adenine to hypoxanthine (Caulfield et al., 1998; Wink et al., 1991). RNOS can cause both single- and double-strand breaks in DNA.
Furthermore, NO inhibits DNA repair proteins and poly-(ADP-ribose) polymerase (PARP), which regulates DNA repair and apoptosis (Sidorkina et al., 2003). Thus, NO induces DNA damage that can lead to carcinogenesis.

NO can promote cancer by enhancing vascularization, which favors growth and metastasis, and by inhibiting apoptosis. NO induces the expression of VEGF in carcinoma cells and suppresses angiostatin and thrombospondin-1, inhibitors of angiogenesis, resulting in promotion of tumor vascularization (Cooke and Losordo, 2002; Dulak et al., 2000). The growth of xenografted murine mammary adenocarcinoma which expresses iNOS is reduced by treatment with iNOS inhibitor (Thomsen et al., 1997). Nitric oxide (NO)-mediated S-nitrosylation of Bcl-2 prevents its ubiquitination and subsequent proteasomal degradation, leading to inhibition of apoptosis. NO-mediated S-nitrosylation and stabilization of Bcl-2 protein was the primary mechanism involved in the malignant transformation of nontumorigenic lung epithelial cells in response to long-term carcinogen exposure (Azad et al.).

5.2 Anti-cancer effects

In contrast to the aforementioned effects of NO, other studies have shown that NO inhibits cell proliferation and induces apoptosis in various cells including cancer cells, in vitro and in vivo (Chawla-Sarkar et al., 2003; Jarry et al., 2004; Kalivendi et al., 2001; Kotamraju et al., 2007; Notas et al., 2006; Peshes-Yaloz et al., 2007; Wang et al., 2003). Nitrosylcobalamin (NO-Cbl), an analog of vitamin B12 that delivers nitric oxide (NO) and exhibits anti-tumor activity; NO-Cbl increases the expression of tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and its receptors, resulting in apoptosis of human tumors (Chawla-Sarkar et al., 2003). The tumor suppressor P53 participates in numerous critical cellular functions including gene transcription, DNA repair, cell cycle control, genomic stability, and apoptosis (Gottlieb and Oren, 1996; Harris, 1996). DNA damage, especially DNA double strand breaks caused by ionizing radiation or other exogenous mutagens, induces p53 protein accumulation and activation, leading to cell cycle arrest during G1/S transition (Huang et al., 1996). High concentrations of nitric oxide (NO), inducing DNA damages, also triggers wild-type p53 protein accumulation and apoptosis (Messmer et al., 1994). In addition, nitric oxide induces death of colon cancer cells through down-regulation of beta-catenin via proteasome-independent degradation (Prevotat et al., 2006). Some report document specific effects of NO in pancreatic cancer. Decker et al. reported that human pancreatic cancer cells engineered to overexpress eNOS show down-regulation of liver metastasis and tumor growth in mice (Decker et al., 2008). Wang et al. established a role of NOS2 in pancreatic cancer growth and metastasis in an animal model. They demonstrated that pancreatic cancer clones expressing low levels of NOS 2 produced tumors in the pancreas which metastasized to the liver, whereas those expressing high levels of NOS 2 did not (Wang et al., 2003).

6. Growth signaling in cancer

Insulin/insulin-like growth factor (IGF) signals play a key role in cancer proliferation and invasion (Bergmann et al., 1995; Furukawa et al., 2005; Kim et al., 2007). Insulin/IGF-I and IGF-II bind to insulin/IGF-I receptors, leading to tyrosine phosphorylation of the cognate
receptors. Insulin receptor substrate (IRS)-1, an adaptor protein, exists mainly in the cytosol, and binds to phosphorylated insulin receptor (IR) and IGF-I receptor (IGF-IR), resulting in the phosphorylation and activation of IRS-1. IRS-1 activates phosphatidylinositol-3 kinase (PI3K), which in turn activates further down-stream components, including Akt/PKB and glycogen synthase kinase (GSK)-3β. Alternatively, phosphorylated and activated IRS-1 can also bind to another adaptor protein, Grb-2, which activates mitogen-activated protein kinase (MAPK), another major insulin/IGF signaling cascade parallel to the PI3K-Akt/PKB pathway (Ito et al., 1996; Tanaka and Wands, 1996). IRS-1 protein expression is detected in several types of cancer, including pancreatic cancer, breast cancer, and hepatic cell carcinoma (Asano et al., 2005; Chang et al., 2002). Thus, insulin/IGF signaling is thought to play a major role in not only metabolic actions, including stimulation of glucose uptake and synthesis of glycogen and protein, but also in cancer viability including proliferation and invasion. IRS-1 is a key molecule in insulin/IGF signaling that transduces a signal from IR/IGF-IR to both PI3K and MAPK pathways (Asano et al., 2005).

Epidermal growth factor (EGF) signaling also plays a key role in cancer proliferation and invasion. EGF binds to EGF receptor (EGFR) and triggers tyrosine phosphorylation of the receptor. Phosphorylated EGFR activates phosphatidylinositol-3-kinase (PI3K), which activates further down-stream components, including Akt. Alternatively, phosphorylated EGFR can also activate the Ras/MEK/ERK pathway, another major EGF signaling cascade parallel to the PI3K/Akt pathway.

In the section to follow, we present our data showing effects of nitric oxide on growth factor signaling.

**Insulin /IGF-I and EGF Signaling**

![Fig. 1. Insulin/IGF-I and EGF signaling](www.intechopen.com)
7. NO regulates growth signaling

7.1 NO regulates insulin signaling in skeletal muscle

Expression of iNOS is elevated in skeletal muscle of patients with type 2 diabetes (Torres et al., 2004) and in high fat diet-induced diabetic mice. Perreault and Marette showed that disruption of the iNOS gene protects against high fat diet-induced insulin resistance in mice (Perreault and Marette, 2001). Furthermore, we demonstrated that disruption of the iNOS gene reverses IRS-1 protein reduction in skeletal muscles of leptin deficient obese mice and NO-donor treatment induces proteasome-dependent IRS-1 degradation in skeletal muscle cells (Sugita et al., 2005). Thus, NO inhibits insulin signaling and is associated with IRS-1 protein degradation, resulting in insulin resistance. This may explain the occurrence of insulin resistance in patients with inflammation or diabetes.

7.2 NO influences insulin/IGF signals in MIAPaCa-2 cells

We examined whether NO influences on insulin/IGF-I signaling in MIAPaCa-2 cells, a pancreatic cancer cell line. Protein expression and phosphorylation were detected by immunoblotting using specific antibodies. SNAP, a NO-donor, inhibited insulin-stimulated tyrosine phosphorylation of IR, IRS-1, the phosphorylation of Akt/PKB at Ser473, and GSK-3β at Ser9. In addition, SNAP inhibited IGF-I-stimulated tyrosine phosphorylation of IGF-IR and IRS-1, phosphorylation of Akt/PKB at Ser473, and GSK-3β at Ser9. Furthermore, SNAP reduced IRS-1 protein expression, although this did not alter the expression of other IGF signaling proteins, including IGF-IR, Akt/PKB, GSK-3β and Erk 1/2 or of β-actin protein. SNAP induced phosphorylation of Erk 1/2 without stimulation by insulin/IGF-I, and enhanced the insulin/IGF-1-stimulated phosphorylation of Erk 1/2; however, SNAP did not influence Erk 1/2 protein expression in MIAPaCa-2 cells (Figure 2A and B).

Fig. 2. NO influences IGF signals in MIAPaCa-2 cells
GSNO, a NO-donor, inhibited IRS-1 protein expression in MCF-7 as well as MIAPaCa-2 cells in a dose-dependent manner, but did not influence IRS-1 protein expression in MB 468 and Panc-1 cells, which exhibited less IRS-1 protein expression (Figure 3A). The proteasome inhibitor, MG132, completely reversed the reduction of IRS-1 protein expression by NO-donors in MIAPaCa-2 cells. Neither GSNO nor MG132 influenced GSK-3β and β-actin protein expression (Figure 3B). To further investigate IRS-1 protein degradation induced by NO-donor, cDNA constructs of IRS-1 full-length, IRS-1 DM1, IRS-1 DM2, and IRS-1 DM3 were produced and sub-cloned into mammalian expression vectors (Figure 3C). MIAPaCa-2 cells were transfected with these expression vectors. GSNO reduced IRS-1 full-length, IRS-1 DM1, and IRS-1 DM3 protein expression, although GSNO did not alter IRS-1 DM2 and β-actin protein expression (Figure 3D). Ubiquitination of wild-type and mutant IRS-1 was detected by immunoprecipitation using anti-Flag antibody followed by immunoblotting with anti-ubiquitin. SNAP induced the ubiquitination of IRS-1 full-length, IRS-1 DM1, and IRS-1 DM3, but did not induce the ubiquitination of IRS-1 DM2 (Figure 3E). These results indicate that NO-donor is capable of inducing ubiquitination at multiple sites in the carboxy-terminus of the IRS-1 protein.

Fig. 3. NO donor downregulates IRS-1 protein expression through proteasome-mediated degradation in MIAPaCa-2 cells

iNOS protein was detected by immunoblotting in Panc-1 cells, a pancreatic cancer-derived cell line. IRS-1 protein expression was significantly increased by 1400 W, an iNOS specific inhibitor, in a dose-dependent manner. Expression of Akt/PKB, β-actin, and Erk 1/2 protein was unaffected by treatment (Figure 4A). GSNO inhibited IRS-1 protein expression, upregulated by 1400W (Figure 4B). Treatment of 1400W enhanced IGF-I-stimulated tyrosine phosphorylation of IRS-1, phosphorylation of Akt/PKB at Ser 473, and GSK-3β at Ser 9 in

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Panc-1 cells. In contrast, 1400 W did not alter IGF-I-stimulated phosphorylation of Erk 1/2 (Figure 4C). These results indicate that endogenous NO produced by iNOS plays a role in insulin/IGF-I signaling.

Fig. 4. iNOS inhibitor 1400W upregulates IRS-1 protein expression and IRS-1/Akt pathway in Panc-1 cells, a pancreatic cancer cell line

Mammalian expression vectors, pCMV Tag 4/IRS-1 full-length, pCMV Tag 4/IRS-1 DM2 and pCMV Tag 4A vector alone, were transfected into MIAPaCa-2 and incubated with G418 for the selection of protein-expressing cells for more than 14 days. Subsequently, the cells expressing high IRS-1 full-length protein or IRS-1 DM2 protein were cloned.

Proliferation of MIAPaCa-2 cells was elevated in a culture medium containing serum or IGF-I, while no proliferation was observed in a culture medium without serum or IGF-I. Proliferation of cells overexpressing full-length IRS-1 was greater than that of vector alone-transfected cells in the culture medium containing 10 % FBS. By contrast, the proliferation of cells expressing IRS-1 DM2 was attenuated compared to cells transfected with full-length IRS-1 or vector alone (Figure 5). The proliferation of cells transfected with full-length IRS-1 was greater compared to that of vector alone-transfected cells in the culture medium containing 100 nM IGF-I without 10% FBS, while IGF-I-stimulated proliferation of IRS-1 DM2-transfected cell was not observed (Figure 5). GSNO (200 µM) significantly reduced the proliferation of vector alone-, IRS-1 full-length-, and IRS-1 DM2-transfected cells in culture medium containing 10 % FBS or IGF-I. To further investigate the role of iNOS in IGF-I-stimulated proliferation, we evaluated the effects of selective iNOS inhibitor, 1400W, in Panc-1 cells cultured with IGF-I in the absence of FBS. Proliferation in Panc-1 cells was not observed in the presence and absence of 1400W (100 µM), when cultured without serum or IGF-I (Figure 6A). 1400W significantly enhanced the proliferation of Panc-1 cells when cultured with 10% FBS (Figure 6B). In the absence of 1400W, IGF-I failed to increase the cell numbers of Panc-1. The combination of IGF-I and 1400W, however, increased the number of Panc-1 cells (Figure 6C). These results provide further evidence for the involvement of downregulation of IGF-I signaling in NO-induced inhibition of cancer cell proliferation.
In vitro invasive potential of MIAPaCa-2 cells and Panc-1 cells was determined using BioCoat Matrigel Invasion Chambers (Becton Dickinson, Bedford, MA). There was no difference between the invasion of vector alone-, IRS-1 full-length-, and IRS-1 DM2-transfected MIAPaCa-2 cells in the absence of the NO-donor. The addition of 200 µM GSNO markedly reduced invasion in vector alone- and IRS-1 full-length-transfected MIAPaCa-2 cells but did not alter invasion in IRS-1 DM2-transfected MIAPaCa-2 cells (Figure 7A). Invasion in Panc-1 cells incubated with 1400W (5 and 100 µM) was significantly greater than that of untreated cells (Figure 7B).

NO-donor treatment leads to several effects on insulin/IGF signaling in pancreatic cancer cells. NO-donor treatment reduced IRS-1 protein expression via proteasome-dependent degradation, and inhibited insulin/IGF-I-stimulated phosphorylation of Akt/PKB and GSK-3β, while enhancing phosphorylation of Erk 1/2 in pancreatic cancer cells (Figure 2, 3, 4). NO-donor inhibited IGF-I-induced phosphorylation of Akt/PKB and GSK-3β in MIAPaCa-2 cells transfected with IRS-1 wild-type or vector, but not in cells transfected with a dominant
negative carboxy-terminal deletion mutants (Tanaka and Wands, 1996) of IRS-1. This indicates the importance of IRS-1 in the inhibition of insulin/IGF signal by NO. IRS-1 expression and IGF-I signaling have important roles in the proliferation and invasion of MIAPaCa-2 cells and Panc-1 cells, consistent with previous reports on other cancer cells (Kim et al., 2007; Shi et al., 2007; Tanaka and Wands, 1996). NO donor inhibited IGF-I signaling, proliferation, and invasion in MIAPaCa-2 cells transfected with IRS-1 full-length or vector. In contrast, treatment with a selective iNOS inhibitor upregulated IRS-1 protein expression and insulin/IGF signaling, resulting in enhanced proliferation and invasion activity in Panc-1 cells. These results indicate that the expression of IRS-1 protein is regulated by endogenous NO production by iNOS as well as by exogenous NO, resulting in the downregulation of IGF-I signaling and the inhibition of cancer proliferation and invasion in MIAPaCa-2 and Panc-1 cells (Figure 4, 5, 6).

Furthermore, the carboxy-terminus as the site responsible for IRS-1 protein degradation by NO, which is located in SH2-containing molecule binding site next the phosphotyrosine binding (PTB) domain was detected. The observation of the ubiquitination and degradation of IRS-1 deletion mutants indicates the possibility that there may be at least two sites responsible for NO donor-induced ubiquitination in the IRS-1 protein. These data had been published in 2010 (Sugita et al.)

In addition, NO inhibits Akt activity directly through post-translational modification, (Yasukawa et al., 2005), which seems to contribute to NO-induced cancer inhibition.

Furthermore, we confirmed that NO-donors down-regulate EGF-stimulated phosphorylation of EGFR and Akt in colon cancer cells (data not shown).

8. Therapeutic prospects

The usefulness of cancer therapy using NO, including iNOS gene therapy and administration of NO-donor, was recently confirmed in animal models (Adams et al., 2008; Kiziltepe et al., 2007; Wang et al., 2004). Consequently, NO therapy has been focused on, and is currently undergoing clinical evaluation for cancer prevention (Ma et al., 2007). This should leads to clinical trials using NO -donors in the near future. Nitroglycerin, a NO-donor, has long been used as a vasodilating, and the safety of nitroglycerin therapies is well established. Nitroglycerin treatment on non-small cell lung cancer is currently planned as a phase II clinical trial. A promising novel class of drugs, nitric oxide-donating NSAIDs (NO-NSAIDs), has been found to be more active than classical NSAIDs against cancer (Rigas and Williams, 2008). The effects of the NO-donating aspirin derivative, NCX 4040, on three human pancreatic adenocarcinoma cell lines were recently described (Capan-2, MIA PaCa-2 and T3M4)(Rosetti et al., 2006). Clinical trials using NO-donors or NO-donating aspirin derivatives are urgently required.

9. References

Adams C, McCarthy HO, Coulter JA, Worthington J, Murphy C, Robson T et al (2008). Nitric oxide synthase gene therapy enhances the toxicity of cisplatin in cancer cells. J Gene Med.
Ambs S, Merriam WG, Ogunfusika MO, Bennett WP, Ishibe N, Hussain SP et al (1998). p53 and vascular endothelial growth factor regulate tumor growth of NOS2-expressing human carcinoma cells. *Nat Med* 4: 1371-6.

Asano T, Yao Y, Shin S, McCubrey J, Abbruzzese JL, Reddy SA (2005). Insulin receptor substrate is a mediator of phosphoinositide 3-kinase activation in quiescent pancreatic cancer cells. *Cancer Res* 65: 9164-8.

Azad N, Iyer AK, Wang L, Lu Y, Medan D, Castranova V et al (2010). Nitric oxide-mediated bcl-2 stabilization potentiates malignant transformation of human lung epithelial cells. *Am J Respir Cell Mol Biol* 42: 578-85.

Beavo JA (1995). Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol Rev* 75: 725-48.

Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M (1995). Insulin-like growth factor I overexpression in human pancreatic cancer: evidence for autocrine and paracrine roles. *Cancer Res* 55: 2007-11.

Biel M, Seeliger M, Pfeifer A, Kohler K, Gerstner A, Ludwig A et al (1999). Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc Natl Acad Sci U S A* 96: 7553-7.

Camp ER, Yang A, Liu W, Fan F, Somcio R, Hicklin DJ et al (2006). Roles of nitric oxide synthase inhibition and vascular endothelial growth factor receptor-2 inhibition on vascular morphology and function in an in vivo model of pancreatic cancer. *Clin Cancer Res* 12: 2628-33.

Caulfield JL, Wishnok JS, Tannenbaum SR (1998). Nitric oxide-induced deamination of cytosine and guanine in deoxynucleosides and oligonucleotides. *J Biol Chem* 273: 12689-95.

Chang Q, Li Y, White MF, Fletcher JA, Xiao S (2002). Constitutive activation of insulin receptor substrate 1 is a frequent event in human tumors: therapeutic implications. *Cancer Res* 62: 6035-8.

Chawla-Sarkar M, Bauer JA, Lupica JA, Morrison BH, Tang Z, Oates RK et al (2003). Suppression of NF-kappa B survival signaling by nitrosylcobalamin sensitizes neoplasms to the anti-tumor effects of Apo2L/TRAIL. *J Biol Chem* 278: 39461-9.

Collier J, Vallance P (1989). Second messenger role for NO widens to nervous and immune systems. *Trends Pharmacol Sci* 10: 427-31.

Cooke JP, Losordo DW (2002). Nitric oxide and angiogenesis. *Circulation* 105: 2133-5.

Decker NK, Abdelmoneim SS, Yaqoob U, Hendrickson H, Hormes J, Bentley M et al (2008). Nitric oxide regulates tumor cell cross-talk with stromal cells in the tumor microenvironment of the liver. *Am J Pathol* 173: 1002-12.

Dulak J, Jozkowicz A, Dembinska-Kiec A, Guevara I, Zdzienicka A, Zmudzinska-Grochot D et al (2000). Nitric oxide induces the synthesis of vascular endothelial growth factor by rat vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 20: 659-66.

Francis SH, Corbin JD (1999). Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. *Crit Rev Clin Lab Sci* 36: 275-328.

Furukawa M, Raffeld M, Mateo C, Sakamoto A, Moody TW, Ito T et al (2005). Increased expression of insulin-like growth factor I and/or its receptor in gastrinomas is associated with low curability, increased growth, and development of metastases. *Clin Cancer Res* 11: 3233-42.
Gao J, Liu X, Rigas B (2005). Nitric oxide-donating aspirin induces apoptosis in human colon cancer cells through induction of oxidative stress. *Proc Natl Acad Sci U S A* 102: 17207-12.

Gottlieb TM, Oren M (1996). p53 in growth control and neoplasia. *Biochim Biophys Acta* 1287: 77-102.

Harris CC (1996). Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. *J Natl Cancer Inst* 88: 1442-55.

Huang LC, Clarkin KC, Wahl GM (1996). Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc Natl Acad Sci U S A* 93: 4827-32.

Ito T, Sasaki Y, Wands JR (1996). Overexpression of human insulin receptor substrate 1 induces cellular transformation with activation of mitogen-activated protein kinases. *Mol Cell Biol* 16: 943-51.

Jarry A, Charrier L, Bou-Hanna C, Devilder MC, Crussaire V, Denis MG *et al* (2004). Position in cell cycle controls the sensitivity of colon cancer cells to nitric oxide-dependent programmed cell death. *Cancer Res* 64: 4227-34.

Kalivendi SV, Kotamraju S, Zhao H, Joseph J, Kalyanaraman B (2001). Doxorubicin-induced apoptosis is associated with increased transcription of endothelial nitric-oxide synthase. Effect of antiapoptotic antioxidants and calcium. *J Biol Chem* 276: 47266-76.

Kaupp UB (1995). Family of cyclic nucleotide gated ion channels. *Curr Opin Neurobiol* 5: 434-42.

Kim HJ, Litzenburger BC, Cui X, Delgado DA, Grabiner BC, Lin X *et al* (2007). Constitutively active type I insulin-like growth factor receptor causes transformation and xenograft growth of immortalized mammary epithelial cells and is accompanied by an epithelial-to-mesenchymal transition mediated by NF-kappaB and snail. *Mol Cell Biol* 27: 3165-75.

Kiziltepe T, Hidestima T, Ishitsuka K, Ocio EM, Raje N, Catley L *et al* (2007). JS-K, a GST-activated nitric oxide generator, induces DNA double-strand breaks, activates DNA damage response pathways, and induces apoptosis in vitro and in vivo in human multiple myeloma cells. *Blood* 110: 709-18.

Kotamraju S, Williams CL, Kalyanaraman B (2007). Statin-induced breast cancer cell death: role of inducible nitric oxide and arginase-dependent pathways. *Cancer Res* 67: 7386-94.

Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S *et al* (1997). A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. *J Biol Chem* 272: 4323-6.

Ma Q, Wang Y, Gao X, Ma Z, Song Z (2007). L-arginine reduces cell proliferation and ornithine decarboxylase activity in patients with colorectal adenoma and adenocarcinoma. *Clin Cancer Res* 13: 7407-12.

Messmer UK, Ankarcrona M, Nicotera P, Brune B (1994). p53 expression in nitric oxide-induced apoptosis. *FEBS Lett* 355: 23-6.

Notas G, Nifli AP, Kampa M, Vercauteren J, Kou roumalis E, Castanas E (2006). Resveratrol exerts its antiproliferative effect on HepG2 hepatocellular carcinoma cells, by inducing cell cycle arrest, and NOS activation. *Biochim Biophys Acta* 1760: 1657-66.
Numajiri N, Takasawa K, Nishiya T, Tanaka H, Ohno K, Hayakawa W et al (2010). On-off system for PI3-kinase-Akt signaling through S-nitrosylation of phosphatase with sequence homology to tensin (PTEN). *Proc Natl Acad Sci U S A* 108: 10349-54.

Palmer RM, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524-6.

Park HS, Huh SH, Kim MS, Lee SH, Choi EJ (2000). Nitric oxide negatively regulates c-Jun N-terminal kinase/stress-activated protein kinase by means of S-nitrosylation. *Proc Natl Acad Sci U S A* 97: 14382-7.

Perreault M, Marette A (2001). Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 7: 1138-43.

Peshes-Yaloz N, Rosen D, Sondel PM, Krammer PH, Berke G (2007). Up-regulation of Fas (CD95) expression in tumour cells in vivo. *Immunology* 120: 502-11.

Prevotat L, Filomenko R, Solary E, Jeannin JF, Bettaieb A (2006). Nitric oxide-induced down-regulation of beta-catenin in colon cancer cells by a proteasome-independent specific pathway. *Gastroenterology* 131: 1142-52.

Reynaert NL, Ckless K, Korn SH, Ves N, Guala AS, Wouters EF et al (2004). Nitric oxide represses inhibitory kappaB kinase through S-nitrosylation. *Proc Natl Acad Sci U S A* 101: 8945-50.

Rigas B, Williams JL (2008). NO-donating NSAIDs and cancer: an overview with a note on whether NO is required for their action. *Nitric Oxide* 19: 199-204.

Rosetti M, Tesei A, Ulivi P, Fabbri F, Vannini I, Brigliadori G et al (2006). Molecular characterization of cytotoxic and resistance mechanisms induced by NCX 4040, a novel NO-NSAID, in pancreatic cancer cell lines. *Apoptosis* 11: 1321-30.

Shi B, Sepp-Lorenzino L, Prisco M, Linsley P, deAngelis T, Baserga R (2007). Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. *J Biol Chem* 282: 32582-90.

Stamler JS, Lamas S, Fang FC (2001). Nitrosylation. the prototypic redox-based signaling mechanism. *Cell* 106: 675-83.

Sugita H, Fujimoto M, Yasukawa T, Shimizu N, Sugita M, Yasuhara S et al (2005). Inducible nitric-oxide synthase and NO donor induce insulin receptor substrate-1 degradation in skeletal muscle cells. *J Biol Chem* 280: 14203-11.

Sugita H, Kaneki M, Furushashi S, Hirota M, Takamori H, Baba H (2010). Nitric oxide inhibits the proliferation and invasion of pancreatic cancer cells through degradation of insulin receptor substrate-1 protein. *Mol Cancer Res* 8: 1152-63.

Tanaka S, Wands JR (1996). A carboxy-terminal truncated insulin receptor substrate-1 dominant negative protein reverses the human hepatocellular carcinoma malignant phenotype. *J Clin Invest* 98: 2100-8.

Thomsen LL, Scott JM, Topley P, Knowles RG, Keerie AJ, Frend AJ (1997). Selective inhibition of inducible nitric oxide synthase inhibits tumor growth in vivo: studies with 1400W, a novel inhibitor. *Cancer Res* 57: 3300-4.

Torres SH, De Sanctis JB, de LBM, Hernandez N, Finol HJ (2004). Inflammation and nitric oxide production in skeletal muscle of type 2 diabetic patients. *J Endocrinol* 181: 419-27.
Wang B, Wei D, Crum VE, Richardson EL, Xiong HH, Luo Y et al (2003). A novel model system for studying the double-edged roles of nitric oxide production in pancreatic cancer growth and metastasis. *Oncogene* 22: 1771-82.

Wang Z, Cook T, Alber S, Liu K, Kovesdi I, Watkins SK et al (2004). Adenoviral gene transfer of the human inducible nitric oxide synthase gene enhances the radiation response of human colorectal cancer associated with alterations in tumor vascularity. *Cancer Res* 64: 1386-95.

Weiss JM, Ridnour LA, Back T, Hussain SP, He P, Maciag AE et al (2010). Macrophage-dependent nitric oxide expression regulates tumor cell detachment and metastasis after IL-2/anti-CD40 immunotherapy. *J Exp Med* 207: 2455-67.

Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM et al (1991). DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 254: 1001-3.

Xie K, Fidler IJ (1998). Therapy of cancer metastasis by activation of the inducible nitric oxide synthase. *Cancer Metastasis Rev* 17: 55-75.

Yasukawa T, Tokunaga E, Ota H, Sugita H, Martyn JA, Kaneki M (2005). S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. *J Biol Chem* 280: 7511-8.
This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyanate and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-flourouracil.

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