

## ENDOGENOUS MORPHINERGIC SIGNALING AND TUMOR GROWTH

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### 1. ABSTRACT

The  $\mu_3$  opiate receptor subtype has been characterized by various binding assays as opiate alkaloid selective (e.g. morphine) and opioid peptide (e.g. methionine enkephalin) insensitive. This opiate receptor subtype has been found on human, including cancer cell lines, and invertebrate tissues, demonstrating that it has been conserved during evolution. Furthermore, in numerous reports, this receptor is coupled to constitutive nitric oxide release. In this regard, for example, morphine immune down regulating activities parallels those actions formerly attributed to nitric oxide. We have now identified the  $\mu_3$  receptor at the molecular level and sequence analysis of the isolated cDNA suggests that it is a novel, alternatively spliced variant of the  $\mu$  opiate receptor gene (MOR). Furthermore, using Northern blot, reverse transcription coupled to polymerase chain reaction (RT-PCR) and sequence analysis, we have demonstrated the expression of this new  $\mu$  variant in human vascular tissue, mononuclear cells, polymorphonuclear cells, and human neuroblastoma cells. The presence of this  $\mu$  splice variant, adds to the growing body of evidence supporting the hypothesis that morphine is an endogenous signaling molecule in neural, immune and vascular systems. In addition to their use in the treatment of pain, opioid peptides appear to be important in the growth regulation of normal and neoplastic tissue. This review will focus on the influence of opiate alkaloids, e.g., morphine, on tumor growth, with emphasis on immuno-regulatory and antiproliferative mechanisms.

### 2. INTRODUCTION

Opium has long been associated with human

history (1). Sumerian ideograms (4,000 B.C.) refer to poppy as the "plant of joy" and Egyptian medical papyri used opium in many of their beneficial medical formulas. It is referred to in Homer's Iliad and Odyssey and in Virgil's Aeneid. The Persian physician Avicenna prescribed it for cough, anemia and diarrhea. Despite this positive medicinal link between man and opium, we all know it has its dark side in substance abuse, culminating in addiction. Based on the historical belief that morphine was not present endogenously in animals, studies on the pharmacologic properties of morphine and morphine-like substances had focused on the effects of exogenous opiates, a family of important analgesic and antinociceptive drugs. This focus changed following the discovery (2) that morphine binds to the same receptors used by endogenous opioid peptides. An important step forward was the demonstration of endogenous opiates in various vertebrate tissues, including the nervous system (3, 4). At this turning point, a quest began to identify the possible roles played by this new group of endogenous messenger molecules under both physiological and pathological conditions.

### 3. ENDOGENOUS MORPHINE PROCESSES

#### 3.1. $\mu_3$ binding

Most of our knowledge of opioid receptors is derived from studies of these receptors in the nervous system. These receptors have been classified as delta,  $\mu$  and kappa, and heterogeneity has been shown for each of these major subtypes (5-11). Amino acid sequences have been derived from cloning of certain delta,  $\mu$  and kappa (neuronal) opioid receptor subtypes (12-15).

## Morphinergic Signaling

**Table 1.** Displacement of  $^3\text{H}$ -dihydromorphine (DHM;  $\text{nmol}\cdot\text{L}^{-1}$ ) by opioid ligands in various human tissue membrane suspensions

LIGAND	Vein	Artery	Atria-E	Granulocytes	Monocytes
Delta-agonist					
DPDPE	>1000	>1000	>1000	1000	>1000
Mu-agonist					
Endomorphin 1	>1000	>1000	>1000	>1000	>1000
Endomorphin 2	>1000	>1000	>1000	>1000	>1000
Orphanin FQ (N)	>1000	>1000	>1000	>1000	>1000
DAMGO	>1000	>1000	>1000	>1000	>1000
Fentanyl	>1000	>1000	>1000	>1000	>1000
Dihydromorphine	$19 \pm 2.1$	$21 \pm 3.0$	$24.3 \pm 3.1$	$18.4 \pm 2.9$	$20.6 \pm 3.8$
Kappa-agonist					
Dynorphin 1-17	>1000	>1000	>1000	>1000	>1000
50-488h	>1000	>1000	>1000	>1000	>1000
U69-593	>1000	>1000	>1000	>1000	>1000
Antagonists					
Naltrexone	$34 \pm 5.1$	$37 \pm 3.8$	$32.6 \pm 3.5$	$3.7 \pm 6.8$	$47.6 \pm 9.3$

One hundred per cent binding is defined as bound  $^3\text{H}$ -DHM in the presence of 10  $\mu\text{M}$  dextrorphan minus bound  $^3\text{H}$ -DHM in the presence of 10  $\mu\text{M}$  levorphanol.  $\text{IC}_{50}$  is defined as the concentration of drug which elicits half-maximal inhibition of specific binding. The mean SD for three experiments is given. DPDPE = (D-Pen<sup>2</sup>, D-Pen<sup>5</sup>)-enkephalin; DAMGO = [Tyr-D-Ala<sup>2</sup>, Gly-N-Me-Phe<sup>4</sup>, Gly(ol)<sup>5</sup>]-enkephalin]

Another mu opiate receptor subtype has been demonstrated, namely  $\mu_3$  (16). The  $\mu_3$  receptor differed from previously described neuronal opioid receptor subtypes in that it exhibited essentially no or exceedingly low affinity for naturally occurring endogenous opioid peptides or their analogues (17). However, the opiate alkaloid binding that was present was naloxone sensitive, demonstrating its opiate receptor properties (18). Also, and of crucial importance, these properties corresponded to effects of opiates, i.e., morphine, on immunocytes that were not mimicked by opioid peptides. In contrast, each of the other opioid receptor subtypes binds at least one of the endogenous opioid peptides with high affinity. Furthermore, certain opiate alkaloids, benzomorphans and other drugs bind to classical opioid receptors but not to the  $\mu_3$  receptor (16, 19-22). Binding sites for this novel morphine (opiate alkaloid-selective, opioid peptide-insensitive) receptor, designated  $\mu_3$ , were first reported to be present in human peripheral blood monocytes and in invertebrate immunocytes and later on other cell types (16, 19, 20, 22-24). The newly discovered opioid peptides endomorphin-1, -2 and orphanin FQ also do not bind to this opiate receptor subtype (table 1) (25). 6-Glucuronide, not the 3-glucuronide metabolite of morphine, binds to the  $\mu_3$  receptor. The potent mu opiate agonist fentanyl does not bind to this receptor as well (20, 22, 23) (table 1).

Classical opioid receptors are linked to trimeric G proteins that in turn modulate  $\text{Ca}^{++}$  and  $\text{K}^+$  channels, adenylyl cyclase, and probably other signal transduction systems (6). In this regard, the  $\mu_3$  receptor is also linked to G protein, based on guanine nucleotide effects on agonist binding to the receptor (20).

### 3.2. Nitric oxide (NO) and morphine

There has been in the literature an association of nitric oxide (NO) with morphine actions. Peripheral morphine analgesia involves NO-stimulated increases in

intracellular cGMP (26). Nitric oxide has been associated with antinociception (27) as well as tolerance and dependence (28). In addition, the morphine-induced suppression of splenic lymphocyte proliferation has been shown to involve NO (29). Morphine and NO have been linked in gastrointestinal regulation (30, 31). Furthermore, morphine, not opioid peptides, stimulate constitutive NO release in macrophages, granulocytes, various types of human and rat endothelial cells, invertebrate neurons and immunocytes and in rat median eminence fragments, all in a naloxone antagonizable manner (18, 25, 32-39). These data suggest that the  $\mu_3$  receptor is coupled to constitutive NO release in these cells.

Furthermore, morphine's actions in these diverse tissues complements what is known about NO mediating immune and vascular functions, namely that it can down regulate them from an excitatory state or prevent the excitatory state from occurring (18, 37, 39, 40). Additional information on opiate alkaloid signaling substances can be summarized as follows: Injection of vertebrate animals with morphine results in deficient macrophage function (41) and an alteration of T-cell activity (42). Morphine also antagonizes interleukin-1 $\alpha$  or tumor necrosis factor- $\alpha$ -induced chemotaxis in human granulocytes and monocytes (43, 44).

### 3.3. Cloning of $\mu_3$

In order to identify  $\mu_3$  at the molecular level, a human testis cDNA library was screened using a MOR-1 gene-specific probe and a 1338 base pair clone was identified (45). Sequencing of this clone and subsequent analysis (NCBI Blast software) shows that the clone exhibits 100% identity to  $\mu_1$  in the center and conserved region, but is truncated at the 5'-end (position 503 of  $\mu_1$  mRNA) (missing several hundred nucleotides). In addition, the 3'-end of the new clone contains the 3'-end of the  $\mu_1$  receptor, followed by a new fragment of 263 bases, and then

5'atacacaagatgaagactgcacacaacatctacatttcaacctgctctggcagatgecttagccaccagtaacctgccccttcagagtgtgaattacctaattgggaacatggccatttgggaaccatcctttgcaagatagtgatctccatagattactataacatgttcaccagcatattcaccctctgcaccatgagtggtgatcgatacttcagctgcaccctgtcaaggccttagatttcgttactcccgaaatgccaaaattatcaatgtctgcaactggatcctctctcagccattggtctctgtaatttcatggtctacaacaaaatacaggcaagggtccatagattgtacactaacattctctcacaacctgggtactgggaaaacctgctgaagatctgtgttttcatctctgcttccattatccagtgctcatcattaccgtgtctatggactgatgattctgcgctcaagagtgtecgatgctctctggctcacaagaaaaggacaggaaattctgaaggatcaccaggatgggtgctggtggtggtgctgtgttcctctgctggaactccattcacattacgtcatcattaaagccttggttacaaaccagaaactacgttcagactgtttctggcactctgcattgctctaggttacacaacacagctgcctcaaccagtcctttatgcattctggaatgaaaactcaaacgctgcttcagagagttctgtatcccaacctcttcaacattgagcaacaaaactcactcgaaattcgtcagaaactagagaccaccctccacggccaatacagtggtatagaactaatcatcagaattattatataattcatagatgttgcgaataccccctcttattctcaaaaagccagctctgctctggtctgattaaagagagaggggtgagtcctgcccactgtgtgtcatgcatgcaagatattcacagaaaattagcatcatagaaaaaaaannnaaaaaaaaaaaaaaaaaancatgctggcgcctcggccaacatcgggtcagcatgcatctaggcgcccaattcggccctctccccccengcnmtttccacaccgaggagtcagtttggcaagacaccagcgggaacaaaacccatcggtgatggaatcgagtcataaaaggtgaccttctgtctgaagatttttaagcatatattatgacctcaacaagacgaaccatctttgttaattcaccgtagtaacacataaagttatgctacctgtatcaaaag-3'

A

MOR-1: 1 \_\_\_\_\_ -2162

MOR-3: 1 \_\_\_\_\_ 1338

B

**Figure 1.** A: Mu3 cDNA sequence (1338 bp). The underlined sequence represents the novel 263 bp segment, and the bold letters represents *Homo sapiens* Oprm 3'UTR DNA sequence (nucleotide position 1625-1829 of Oprm). B: The diagram shows the mu3 cDNA sequence starting at position 503 of Oprm. The dotted lines represent the novel 263 bp mu3 sequence (between nucleotides 1376 and 1625 of Oprm), and position 1625-1829 is the Oprm 3'UTR DNA sequence that represents the 3'-end of mu3.

a 202 bp fragment of the 3'-end of the mu<sub>1</sub> gene untranslated region (45). RT-PCR and subsequent sequence analysis revealed the presence of this opiate receptor subtype in human vascular endothelial cells, mononuclear cells and polymorphic nuclear cells (45) (see sequence in figure 1).

To determine if the cDNA clone we isolated was functional and had the biochemical properties expected of the mu<sub>3</sub> receptor, the cDNA was expressed in a heterologous system (Cos-1 cells). Following exposure to morphine, the transfected Cos-1 cells released NO in a naloxone antagonizable manner (45). Untransfected Cos-1 cells failed to produce any detectable NO upon addition of morphine (45). The addition of Met-enkephalin, DPDPE, or Leu-enkephalin did not stimulate NO release in the controls or transfected cells (45). These results show that transfection of the novel cDNA clone isolated from human testes conferred the expected opiate alkaloid selective and opioid peptide insensitive characteristics of the mu<sub>3</sub> opiate receptor (17, 46-48). In addition, we analyzed human heart endothelial tissue, human leukocytes, and Cos-1 transfected cells for expression of the mu<sub>3</sub> mRNA by Northern blot analysis (45). A band of the expected size of approximately 1.45 kb was observed in the various tissues (45). The selectivity of the mu<sub>3</sub> opiate receptor subtype, therefore, provides further evidence for the status of morphine as an endogenous signaling molecule (46).

### 3.4. Endogenous morphine

The demonstration of endogenous opiates, i.e., morphine, codeine, in various vertebrate tissues, including the nervous system (3, 40, 49-53), is quite important for establishing the significance of the mu<sub>3</sub> opiate receptor subtype. There is a body of evidence that shows opiate alkaloids such as morphine, morphine-3- and 6-glucuronide, as well as the morphine putative precursor

molecules (thebaine, salutaridine, norcoccularine, reticuline, tetrahydropapaverine (THP) and codeine) exist in vertebrates (52, 54-56). In invertebrates, specifically *Mytilus edulis*, the presence of morphine, morphine 6-glucuronide, morphine 3-glucuronide, codeine, THP and reticuline also have been reported (16, 46, 57-59). Endogenous opiate levels can be induced to change following stimulation (46). Morphine has also been found in human plasma (60, 61), suggesting a hormonal action with immune, vascular and gut tissues as targets (31, 62). Additionally, in recent studies sources of contamination for these compounds were examined, i.e., rat food, supplies, etc, and not found (56). Besides these biochemical studies, immunocytochemical localization of a morphine-like material was reported in neural and immune tissues (63-66) as well as in invertebrate tissues (16). Taken together, these reports suggest that animals appear to have the ability to synthesize opiate alkaloids.

In earlier reports we have formulated a hypothesis, which states that endogenous morphine, used as either a hormone or neurotransmitter down regulates immune, vascular, neural and gut tissues under normal and following trauma situations (18, 31, 40, 46, 66). In this regard, as noted in these reports, tolerance is viewed as a mechanism to ensure that morphine's continued presence does not permanently limit a needed tissue excitatory state, since excitation may be required to overcome a traumatic event, which morphine would continue to down regulate.

## 4. MORPHINE AND TUMOR GROWTH

### 4.1. Effects of morphine on tumor growth

Opioid peptides, such as methionine enkephalin, have been implicated in a wide variety of pharmacological and physiological functions (18). In addition to their use in the treatment of pain, opioid peptides appear to be

important in the growth regulation of normal and neoplastic tissue. This literature regarding opioid peptides and tumor growth has been reviewed recently (67). This portion of the review will focus on the influence of endogenous and exogenous opiate alkaloids, e.g., morphine, on tumor growth, with emphasis on immunoregulatory and antiproliferative mechanisms. This has become important because morphinergic signaling appears to be an endogenous messenger system, as noted in the earlier discussion. Additionally, most of the reports that will be discussed examine morphine signaling from an exogenous perspective, leaving open the issue of how endogenous morphine impacts tumor growth. This article will focus on morphine's influence on the regulatory mechanisms involved in tumor growth.

Opiate alkaloids appear to represent one of the immune and vascular inhibitory/anti-inflammatory systems in an organism whereas opioid peptides appear to have proinflammatory capabilities (18, 46, 68). Thus, from an immune perspective, these signaling molecules are potential candidates as tumor growth modifiers.

### 4.2. Exogenous morphine affects tumor growth

Morphine is widely used as an analgesic to treat pain in a variety of patients, including those with cancer. There is evidence that morphine has extra-analgesic actions and significantly alters tumor growth. Ishikawa *et al* demonstrated that morphine (10 mg/kg) given daily for 10 days enhanced the growth of several different tumor cell lines *in vivo* (69). However, other studies suggest that the analgesic qualities of morphine contribute to the control of metastasis following surgery. Page *et al* demonstrated that pre- and postoperative administration of morphine significantly attenuated the metastatic-enhancing effects of surgery (70-72). In addition, intermittent bolus morphine administration to animals was associated with a reduction in the growth of tumor cells that gained access to the circulation during the surgical procedure (73). The authors proposed that the most likely explanation was a direct morphine effect on host resistance to metastatic tumor growth.

Hatzoglou *et al* demonstrated that morphine decreases the cell growth of human breast cancer cells *in vitro*, despite the lack of mu receptors in the cancer cells as determined by a limited screening with pharmacological agents (74). They raised the question whether this antiproliferative effect of morphine could be mediated through interaction with other receptor systems. In a further study they showed, that morphine may exert its antiproliferative effect on breast cancer cells through interaction with the somatostatin receptor SSTR2, suggesting a functional interaction of morphine with the inhibitory somatostatinergic system (75). Previous studies have demonstrated a direct inhibitory effect of somatostatin analogues on the growth of human cancer cells (76-78). The possible interaction of morphine with other receptor systems is supported by the findings of Maneckjee and Minna. They found that the inhibitory effects of morphine on the growth of lung cancer could be reversed by nicotine, suggesting an interaction between the opioid system and

acetylcholine receptors (79). In addition, Zagon *et al* demonstrated in receptor binding studies that only a few tumors express mu opioid binding sites (80). Thus, given the knowledge at that time, it seems that mu opioid receptors do not play a significant role in opioid mediated regulation of tumor growth. However, new evidence from our group suggests that other physiologically active mu opioid receptor splice variants may be present and operational in immune and vascular tissues that had gone previously undetected (45, 48). Thus, morphine may be protective in many tissues in response to stress or injuries (46) and it is no surprise that in cases where there is tissue injury such as in wound healing it is involved in angiogenesis (81). In part, this effect may be due to rebound excitation, following morphine's immediate action, which is down regulating in nature (37, 40, 46). This rebound excitation allows tissues, i.e., immune, a chance to exhibit enhanced surveillance, following morphine stimulated immune suppression (82, 83). Clearly, this action has important organism survival value, which can also be surmised by the fact that this process is found in organisms that evolved 500 million years before man (82).

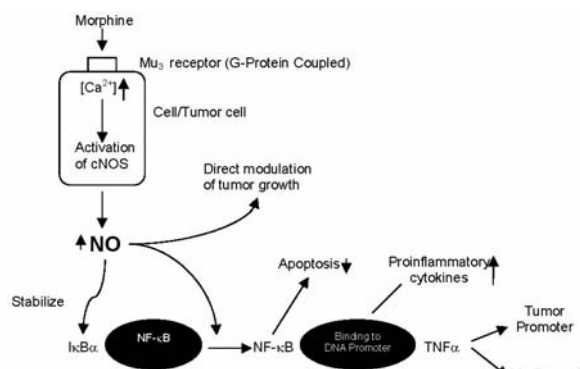
### 4.3. Morphine and apoptosis

The molecular mechanism by which morphine influences tumor growth *in vivo* and *in vitro* is not clear. Basically, this situation exists because of a lack of realization that morphine represents an endogenous signaling system (40, 46, 56). However, one hypothesis is that morphine promotes apoptosis in tumor cells. Maneckjee and Minna demonstrated that treatment of human lung cancer cells with 0.1-1.0 uM morphine or methadone resulted in morphological changes and cleavage of DNA into nucleosome-sized fragments characteristic of apoptosis (84). Sueoka *et al* demonstrated that morphine attenuated the growth of various cancer cell lines *in vitro* through inhibition of tumor necrosis factor (TNF)-alpha mRNA expression and TNF-alpha release (85). Transcription of TNF-alpha gene is in part regulated by the transcription factor, nuclear factor kappa B (NFkB) (86). In a further study, they demonstrated that the anticancer activity of morphine and the five times more potent morphine derivatives, (-)-3-Acetyl-6beta-acetylthio-N-cyclopropyl-methylnormorphine (KT-90), (-)-6beta-acetylthiomorphine (KT 87) was mediated through apoptosis associated with inhibition of NFkB in human cancer cell lines (87). NFkB is a DNA binding protein that induces expression of genes for several inflammatory mediators such as TNF-alpha and augments transcription of various genes involved in cell proliferation. Recently it was demonstrated that inhibition of NFkB attenuates apoptosis resistance in lymphoid cells (88). Furthermore, we demonstrated that morphine can directly inhibit NFkB actions via the liberation of NO (89, 90), introducing another variable in the morphine signaling cascade that may explain morphine's antiproliferative and apoptotic actions (figure 2).

### 4.4. Morphine influences natural killer (NK) cell activity

The role of NK cells in both the metastasis enhancing effects of surgery and the attenuation of these effects by morphine was investigated by Page *et al* (71). Surgery induced a suppression of whole blood NK cytotoxic

## Morphinergic Signaling



**Figure 2.** How morphine might alter tumor growth. Morphine stimulates intracellular  $\text{Ca}^{2+}$  transients that, in turn, activate constitutive nitric oxide synthase (cNOS) and liberate nitric oxide (NO). This modulation occurs via the  $\mu_3$  opiate receptor subtype that is G protein regulated. NO may have a direct effect on tumor growth. Furthermore, NO inhibits disassociation of the I $\kappa$ B $\alpha$  inhibitor complex, NF- $\kappa$ B binding to the respective DNA promoter region and the subsequent expression of inflammatory cytokines, thereby stimulating apoptosis and downregulation of the carcinogenic effects of TNF- $\alpha$ .

cytotoxic activity and a decreased number of circulating Large Granular Lymphocytes (LGL)/NK cells measured 4 h postoperatively (71). The authors observed that in LGL/NK depleted animals, morphine had no impact on tumor cell retention. They suggested that LGL/NK cells play a critical role in morphine's antimetastatic effects. In addition, Provincially *et al* reported that "chronic" morphine treatment in cancer patients was accompanied by a decrease in the *in vitro* NK cell cytotoxic activity (91) while the treatment significantly increased Lymphokine Activated Killer (LAK) cell activity when compared to healthy controls (91).

### 4.5. Morphine's influences on DNA synthesis

It has previously been demonstrated that  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid agonists attenuate thymidine incorporation into DNA in glial (92) and developing neural cells (80, 93-96). Furthermore, Barg *et al* showed that morphine inhibited DNA synthesis through inhibition of thymidine incorporation in C6 rat glioma cells that express opioid receptors (97). Their results imply that inhibition of phosphoinositol signal transduction and  $\text{Ca}^{2+}$  mobilization is responsible for reduction in thymidine incorporation (98). This finding is in accordance with previous reports demonstrating that opioid peptides and opiate alkaloids can inhibit phosphoinositol turnover (92, 99-101).

### 4.6. Endogenous morphine, nitric oxide and the regulation of tumor growth

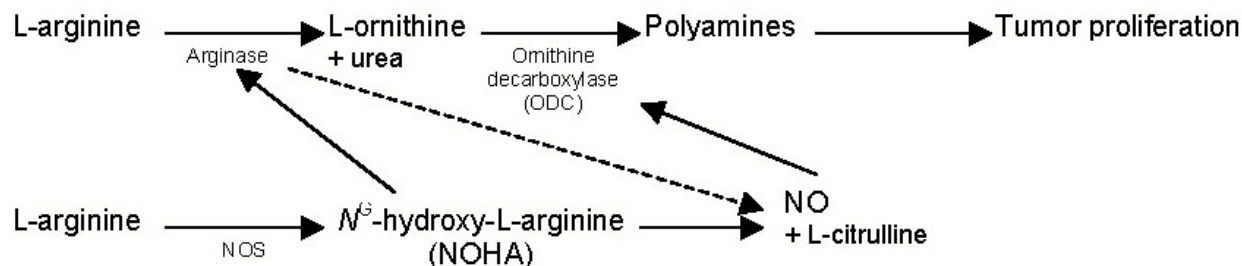
Morphine has been shown to be involved in immunomodulation. Studies have demonstrated that morphine, not opioid peptides, via the  $\mu_3$  opiate receptor is coupled to constitutive NO release in endothelial and immunocompetent cells (22, 45, 102, 103). Recently, expression of this opiate receptor subtype was demonstrated in human specimens of cancer tissue (non-

small-cell lung carcinoma) (104). The authors demonstrated that activation of the  $\mu_3$  opiate receptor by opiate alkaloids in tumor cells leads to a rapid and substantial release of NO (104). They suggested that the anti cancer effects of morphine were mediated by NO through the  $\mu_3$  receptor. They also speculated that increased NO production in lung carcinoma may indicate that tumors use endogenous opiates that bind to the  $\mu_3$  receptor and thereby down regulate the host immune response to tumor growth.

Based on the earlier discussion, concerning endogenous morphine and the  $\mu_3$  opiate receptor's coupling to cNOS-derived NO we surmise this NO, in turn, inhibits binding of the DNA transcription factor NF $\kappa$ B to DNA and thereby down regulates the expression of genes for several inflammatory cytokines (46, 89, 90, 104, 105) (figure 2). Furthermore, NO stabilizes the NF $\kappa$ B inhibitor, Inhibitory kappa B alpha (I $\kappa$ B $\alpha$ ), which prevents its degradation. In addition, inhibition of NF $\kappa$ B attenuates apoptosis resistance as described previously and with that promotes down regulation of tumor growth (figure 2).

Regarding the effect of NO on tumor growth regulation, previous studies have shown that NO produced by immune and endothelial cells is tumoricidal possibly by inducing apoptosis (106). Other studies have demonstrated that up regulation of the expression of inducible NO synthase (iNOS) in hepatic metastases and metastatic melanoma cells is associated with apoptosis, suppression of tumorigenicity, and abrogation of metastasis (107, 108). However, other studies have demonstrated tumor promoting effects of NO, and overall, NO seems to play a variety of contradictory roles in tumor growth regulation (109, 110). Contradicting results have also been obtained regarding the functional interaction between the opioidergic and the NO system. Kampa *et al* demonstrated that opioid agonists, active on kappa-opioid receptors decrease  $\text{NO}_2^-/\text{NO}_3^-$  release and NOS activity *in vitro* (111). Based on their results and reports demonstrating NO involvement in tumor progression and metastasis, the authors suggested opiates as a potential intervention in cancer treatment. Furthermore, ambiguity is introduced into this field because most research fails to take into account the rebound effect that occurs following exposure of morphine to these cells (37, 38, 82, 83, 112). Here we've surmised, since morphine is endogenous, that following immediate morphine exposure processes are down regulated, then after some time (hours), their activity returns but to a higher level for a short period of time. This secondary immune and vascular enhancement of activity, we surmise, is designed to take care of any emergent problems that occurred during the period of down regulation, offering yet another mechanism that enhances the survival of such endowed organisms (37, 38, 82, 83, 112).

Recently, Gobert *et al* demonstrated that the vigorous host response, i.e., up regulation of iNOS, to the human gastric pathogen *Helicobacter pylori* failed to eradicate the organism (113). This was due to bacterial arginase down-regulating eukaryotic NO production. We surmise a similar process may be occurring with tumors,



**Figure 3. The mechanisms by which L-arginine metabolites influence cell proliferation.** L-arginine is the common substrate for two enzymes, arginase and nitric oxide synthase (NOS). Arginase converts L-arginine to L-ornithine. L-ornithine can be subsequently used by ODC to form polyamines, which are essential for cell proliferation. NO is a potent inhibitor of ODC, suggesting that the antiproliferative role of NO is attributed to inhibition of polyamine formation. L-arginine is also metabolized by NOS to L-citrulline and NO via an intermediate, NOHA. NOHA interferes with cell proliferation by inhibition of arginase and arginine-derived polyamines. Arginase promotes polyamine formation and suppresses tumor cytotoxicity by reducing NO production.

explaining the contradictory results. That is, certain tumors have a process to neutralize NO tumoricidal actions. In this regard the generation of NO through activation of NOS has been shown to be antiproliferative (114). However, the mechanism by which increases in NOS activity and the production of NO causing cytostasis is not clear. The intermediate *N*-omega-hydroxy-L-arginine (NOHA) in the oxidation of arginine is a strong inhibitor of the enzyme arginase. One of the products of arginase catalyzing L-arginine is L-ornithine. L-ornithine can be subsequently used by the enzyme ornithine decarboxylase (ODC) to form polyamines, which are essential components of cell proliferation (figure 3). Cells that have been activated with cytokines show a significant increase in NOHA and NO or its oxidized metabolites (114). Therefore, NOHA may be playing a role as a biological inhibitor of endogenous arginase activity, thereby promoting down regulation of tumor cell proliferation (figure 3). This may explain the mechanism by which activation of NOS is involved in cytostasis. In addition it has been demonstrated that NO is a potent inhibitor of ODC, thereby suggesting that the antiproliferative action of NO is attributed to inhibition of polyamine formation (114) (figure 3).

## 5. PERSPECTIVES

Few breast cancer cells lines were shown to have high arginase activity and very low NOS activity (115). Nitric oxide derived from macrophages is known to have tumoricidal activity and polyamines may promote the growth of tumor cells (116). Therefore, it appears that arginase may be playing a role in promoting tumor growth by inhibiting the production of NO. The significance of this phenomenon is enhanced by our early discussion concerning morphine's ability to stimulate cNOS derived NO release, since this action may not be observed in the presence of arginase. In this regard, a biomedical strategy recognizing this process may be designed, strengthening a role for opiate as a new tumoricidal agent.

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## 7. REFERENCES

- Merlin, M. D: On The Trail of Ancient Opium Poppy. (1984)
- Lord, J. A. H., A. A. Waterfield, J. Hughes and H. W. Kosterlitz: Endogenous opioid peptides: Multiple agonists and receptors. *Nature* 267, 495-499 (1977)
- Gintzler, A. R., A. Levy and S. Spector: Antibodies as a means of isolating and characterizing biologically active substances: Presence of a non-peptide morphine-like compound in the central nervous system. *Proc Nat Acad Sci USA* 73, 2132-2136 (1976)
- Gintzler, A. R., M. D. Gershon and S. Spector: A nonpeptide morphine-like compound: immunocytochemical localization in the mouse brain. *Science* 199, 447-448 (1978)
- Pasternak, G. W: Multiple mu opiate receptors: Biochemical and pharmacological evidence for multiplicity. *Biochem Pharmacol* 35, 361-364 (1986)
- Pasternak, G. W: The Opiate Receptors. (1988)
- Clark, J. A., L. Liu, M. Price, B. S. Hersh, M. Edelson and G. W. Pasternak: Kappa opiate receptor multiplicity: Evidence for two U50, 488- sensitive K1 subtypes and a novel K3 subtype. *J Pharmacol Exp Ther* 251, 461-468 (1989)
- Rothman, R. B., V. Bykov, B. R. DeCosta, A. E. Jacobson, K. C. Rice and L. S. Brady: Interaction of endogenous opioid peptides and other drugs with four kappa binding sites in guinea pig brain. *Peptides* 11, 311-331 (1990)
- Mattia, A., S. C. Farmer, A. E. Takemori, M. Sultana, P. S. Portoghese, H. I. Mosberg, W. D. Bowen and F. Porreca: Spinal opioid *delta* antinociception in the mouse mediation by a 5'-NTII-sensitive *delta* receptor subtype. *J Pharmacol*

*Exp Ther* 260, 518-525 (1992)

10. Stefano, G. B., P. Melchiorri, L. Negri, T. K. Hughes and B. Scharer: (D-Ala<sup>2</sup>)-Deltorphin I binding and pharmacological evidence for a special subtype of delta opioid receptor on human and invertebrate immune cells. *Proc Natl Acad Sci USA* 89, 9316-9320 (1992)

11. Traynor, J. R. and J. Elliott: Delta-opioid receptor subtypes and cross-talk with mu-receptors. *Trends Pharmacol Sci* 14, 84-86 (1993)

12. Kieffer, B. L., K. Befort, C. E. Gaveriaux-Ruff and C. G. Hirth: The delta-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. *Proc Natl Acad Sci USA* 89, 12048-12052 (1992)

13. Evans, C. J., D. E. Keith, Jr., H. Morrison, K. Magendzo and R. H. Edwards: Cloning of a delta opioid receptor by functional expression. *Science* 258, 1952-1955 (1992)

14. Chen, Y., A. Mestek, J. Liu, J. A. Hurley and L. Yu: Molecular cloning and functional expression of a mu-opioid receptor from rat brain. *Mol Pharmacol* 44, 8-12 (1993)

15. Yasuda, K., K. Raynor, H. Kong, C. D. Breder, J. Takeda, T. Reisine and G. I. Bell: Cloning and functional comparison of kappa and delta-opioid receptors from mouse brain. *Proc Natl Acad Sci USA* 90, 6736-6740 (1993)

16. Stefano, G. B., A. Digenis, S. Spector, M. K. Leung, T. V. Bilfinger, M. H. Makman, B. Scharer and N. N. Abumrad: Opiatolike substances in an invertebrate, a novel opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. *Proc Natl Acad Sci USA* 90, 11099-11103 (1993)

17. Stefano, G. B.: The mu<sub>3</sub> opiate receptor subtype. *Pain Forum* 8, 206-209 (1999)

18. Stefano, G. B., B. Scharer, E. M. Smith, T. K. Hughes, H. I. Magazine, T. V. Bilfinger, A. Hartman, G. L. Fricchione, Y. Liu and M. H. Makman: Opioid and opiate immunoregulatory processes. *Crit Rev in Immunol* 16, 109-144 (1996)

19. Cruciani, R. A., B. Dvorkin, H. P. Klinger and M. H. Makman: Presence in neuroblastoma cells of a mu<sub>3</sub> receptor with selectivity for opiate alkaloids but without affinity for opioid peptides. *Brain Res* 667, 229-237 (1994)

20. Makman, M. H., T. V. Bilfinger and G. B. Stefano: Human granulocytes contain an opiate receptor mediating inhibition of cytokine-induced activation and chemotaxis. *J Immunol* 154, 1323-1330 (1995)

21. Makman, M. H., B. Dvorkin and G. B. Stefano: Murine macrophage cell lines contain mu<sub>3</sub>-opiate receptors. *Eur J*

*Pharmacol* 273, R5-R6 (1995)

22. Stefano, G. B., A. Hartman, T. V. Bilfinger, H. I. Magazine, Y. Liu, F. Casares and M. S. Goligorsky: Presence of the mu<sub>3</sub> opiate receptor in endothelial cells: Coupling to nitric oxide production and vasodilation. *J Biol Chem* 270, 30290-30293 (1995)

23. Dobrenis, K., M. H. Makman and G. B. Stefano: Occurrence of the opiate alkaloid-selective mu<sub>3</sub> receptor in mammalian microglia, astrocytes and kupffer cells. *Brain Res* 686, 239-248 (1995)

24. Stefano, G. B. and B. Scharer: The presence of the mu<sub>3</sub> opiate receptor in invertebrate neural tissues. *Comp Biochem Physiol* 113C, 369-373 (1996)

25. Rialas, C., T. V. Bilfinger, M. Salzet and G. B. Stefano: Endomorphin 1 and 2 do not interact with the mu<sub>3</sub> opiate receptor subtype. *Acta Pharmacol Sin* 19, 403-407 (1998)

26. Ferreira, S. H., I. D. Duarte and B. B. Lorenzetti: The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release. *Eur J Pharmacol* 201, 121 (1991)

27. Przewlocki, R., H. Machelska and B. Przewlocka: Inhibition of nitric oxide synthetase enhances morphine antinociception in the rat spinal cord. *Life Sci* 53, PL1-5 (1993)

28. Majeed, N. H., B. Przewlocka, H. Machelska and R. Przewlocki: Inhibition of nitric oxide synthase attenuates the development of morphine tolerance and dependence in mice. *Neuropharmacol* 33, 189 (1994)

29. Fecho, K., K. A. Maslonek, M. E. Coussons-Read, L. A. Dykstra and D. T. Lysle: Macrophage-derived nitric oxide is involved in the depressed concanavalin A responsiveness of splenic lymphocytes from rats administered morphine *in vivo*. *J Immunol* 152, 5845-5851 (1994)

30. Gyires, K.: The role of endogenous nitric oxide in the gastroprotective action of morphine. *Eur J Pharmacol* 255, 33-37 (1994)

31. Stefano, G. B., W. Zhu, P. Cadet and K. Mantione: Morphine enhances nitric oxide release in the mammalian gastrointestinal tract via the mu<sub>3</sub> opiate receptor subtype: A hormonal role for endogenous morphine. *Journal of Physiology and Pharmacology* 55, 279-288 (2004)

32. Bilfinger, T. V., A. Hartman, Y. Liu, H. I. Magazine and G. B. Stefano: Cryopreserved veins used for myocardial revascularization: A 5 year experience and a possible mechanism for their increased failure. *Ann Thorac Surg* 63, 1063-1069 (1997)

33. Sawada, M., M. Ichinose and G. B. Stefano: Nitric oxide inhibits the dopamine-induced K<sup>+</sup> current via guanylate cyclase in Aplysia neurons. *J Neurosci Res* 50,



450-456 (1997)

34. Stefano, G. B., B. Salzet, C. M. Rialas, M. Pope, A. Kustka, K. Neenan, S. C. Pryor and M. Salzet: Morphine and anandamide stimulated nitric oxide production inhibits presynaptic dopamine release. *Brain Res* 763, 63-68 (1997)

35. Bilfinger, T. V., C. Fimiani and G. B. Stefano: Morphine's immunoregulatory actions are not shared by fentanyl. *Int J Cardiol* 64, 61-66 (1998)

36. Prevot, V., C. Rialas, D. Croix, M. Salzet, J.-P. Dupouy, P. Puolain, J. C. Beauvillain and G. B. Stefano: Morphine and anandamide coupling to nitric oxide stimulated GnRH and CRF release from rat median eminence: neurovascular regulation. *Brain Res* 790, 236-244 (1998)

37. Stefano, G. B.: Autoimmunovascular regulation: morphine and anandamide stimulated nitric oxide release. *Journal of Neuroimmunology* 83, 70-76 (1998)

38. Stefano, G. B., M. Salzet and T. V. Bilfinger: Long-term exposure of human blood vessels to HIV gp120, morphine and anandamide increases endothelial adhesion of monocytes: Uncoupling of nitric oxide. *J Cardiovasc Pharmacol* 31, 862-868 (1998)

39. Stefano, G. B., M. Salzet, H. I. Magazine and T. V. Bilfinger: Antagonist of LPS and IFN-gamma induction of iNOS in human saphenous vein endothelium by morphine and anandamide by nitric oxide inhibition of adenylate cyclase. *J Cardiovasc Pharmacol* 31, 813-820 (1998)

40. Stefano, G. B. and B. Scharrer: Endogenous morphine and related opiates, a new class of chemical messengers. *Adv Neuroimmunol* 4, 57-68 (1994)

41. Tubaro, E., G. Borelli, C. Croce, G. Cavallo and C. Santiangeli: Effect of morphine on resistance to infection. *J Infect Dis* 148, 656-666 (1983)

42. Weber, R. J., L. C. Band, B. DeCosta, A. Pert and K. C. Rice: Neural control of immune function: Opioids, opioid receptors and immunosuppression. *NIDA Res Monogr* 105, 96-102 (1991)

43. Perez-Castrillon, J. P., J. P. Perez-Arellano, J. D. Garcia-Palomo, A. Jimenez-Lopez and S. DeCastro: Opioids depress *in vitro* human monocyte chemotaxis. *Immunopharmacol* 23, 57-61 (1992)

44. Stefano, G. B. and T. V. Bilfinger: Human neutrophil and macrophage chemokinesis induced by cardiopulmonary bypass: Loss of DAME and IL-1 chemotaxis. *J Neuroimmunol* 47, 189-198 (1993)

45. Cadet, P., K. J. Mantione and G. B. Stefano: Molecular identification and functional expression of mu3, a novel alternatively spliced variant of the human mu opiate receptor gene. *Journal of Immunology* 170, 5118-5123

(2003)

46. Stefano, G. B., Y. Goumon, F. Casares, P. Cadet, G. L. Fricchione, C. Rialas, D. Peter, D. Sonetti, M. Guarna, I. Welters and E. Bianchi: Endogenous morphine. *Trends in Neurosciences* 9, 436-442 (2000)

47. Cadet, P. and G. B. Stefano: *Mytilus edulis* pedal ganglia express mu opiate receptor transcripts exhibiting high sequence identity with human neuronal mu1. *Mol Brain Res* 74, 242-246 (1999)

48. Cadet, P., T. V. Bilfinger, C. Fimiani, D. Peter and G. B. Stefano: Human vascular and cardiac endothelia express mu opiate receptor transcripts. *Endothelium* 7, 185-191 (2000)

49. Goldstein, A., R. W. Barrett, I. F. James, L. I. Lowney, C. Weitz, L. I. Knipmeyer and H. Rapoport: Morphine and other opiates from beef brain and adrenal. *Proc Natl Acad Sci USA* 82, 5203-5207 (1985)

50. Cardinale, G. J., J. Donnerer, A. D. Finck, J. D. Kantrowitz, K. Oka and S. Spector: Morphine and codeine are endogenous components of human cerebrospinal fluid. *Life Sci* 40, 301-306 (1987)

51. Donnerer, J., G. Cardinale, J. Coffey, C. A. Lisek, I. Jardine and S. Spector: Chemical characterization and regulation of endogenous morphine and codeine in the rat. *J Pharmacol Exp Ther* 242, 583-587 (1987)

52. Donnerer, J., K. Oka, A. Brossi, K. C. Rice and S. Spector: Presence and formation of codeine and morphine in the rat. *Proc Natl Acad Sci USA* 83, 4566-4567 (1986)

53. Oka, K., J. D. Kantrowitz and S. Spector: Isolation of morphine from toad skin. *Proc Natl Acad Sci USA* 82, 1852-1854 (1985)

54. Lee, C. S. and S. Spector: Changes in endogenous morphine and codeine contents in the fasting rat. *Journal of Pharmacology and Experimental Therapeutics* 257, 647-652 (1991)

55. Epple, A., B. Nibbio, S. Spector and J. Brinn: Endogenous codeine: Autocrine regulator of catecholamine release from chromaffin cells. *Life Sci* 54, 695-702 (1994)

56. Zhu, W., Y. Ma, P. Cadet, D. Yu, T. V. Bilfinger, E. Bianchi and G. B. Stefano: Presence of reticuline in rat brain: A pathway for morphine biosynthesis. *Mol Brain Res* 117, 83-90 (2003)

57. Goumon, Y., F. Casares, W. Zhu and G. B. Stefano: The presence of morphine in ganglionic tissues of *Modiolus demissus*: A highly sensitive method of quantitation for morphine and its derivatives. *Mol Brain Res* 86, 184-188 (2001)

58. Zhu, W., G. Baggerman, Y. Goumon, F. Casares, B. Brownwell and G. B. Stefano: Presence of morphine and



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morphine-6-glucuronide in the marine mollusk *Mytilus edulis* ganglia determined by GC/MS and Q-TOF-MS. Starvation increases opiate alkaloid levels. *Mol Brain Res* 88, 155-160 (2001)

59. Zhu, W., Y. Ma and G. B. Stefano: Presence of isoquinoline alkaloids in molluscan ganglia. *Neuroendocrinol Lett* 23, 329-334 (2002)

60. Brix-Christensen, V., E. Tonnesen, R. G. Sanchez, T. V. Bilfinger and G. B. Stefano: Endogenous morphine levels increase following cardiac surgery as part of the antiinflammatory response? *Int J Cardiol* 62, 191-197 (1997)

61. Cadet, P., W. Zhu, K. Mantione, M. Rymer, I. Dardik, S. Reisman, S. Hagberg and G. B. Stefano: Cyclic exercise induces anti-inflammatory signal molecule increases in the plasma of Parkinson's patients. *Int J Mol Med* 12, 485-492 (2003)

62. Stefano, G. B., W. Zhu, P. Cadet, K. Mantione, T. V. Bilfinger, E. Bianchi and M. Guarna: A hormonal role for endogenous opiate alkaloids: Vascular tissues. *Neuroendocrinology Letters* 23, 21-26 (2002)

63. Bianchi, E., C. Alessandrini, M. Guarna and A. Tagliamonte: Endogenous codeine and morphine are stored in specific brain neurons. *Brain Res* 627, 210-215 (1993)

64. Bianchi, E., M. Guarna and A. Tagliamonte: Immunocytochemical localization of endogenous codeine and morphine. *Adv Neuroimmunol* 4, 83-92 (1994)

65. Liu, Y., T. V. Bilfinger and G. B. Stefano: A rapid and sensitive quantitation method of endogenous morphine in human plasma. *Life Sci* 60, 237-243 (1996)

66. Esch, T., M. Guarna, E. Bianchi, W. Zhu and G. B. Stefano: Commonalities in the central nervous system's involvement with complementary medical therapies: Limbic morphinergic processes. *Medical Science Monitor* (2004)

67. Rasmussen, M., W. Zhu, J. Tonnesen, P. Cadet, E. Tonnesen and G. B. Stefano: Effects of morphine on tumour growth. *Neuroendocrinology Letters* 23, 193-198 (2002)

68. Stefano, G. B., B. Salzet and G. L. Fricchione: Enkefalin and opioid peptide association in invertebrates and vertebrates: immune activation and pain. *Immunol Today* 19, 265-268 (1998)

69. Ishikawa, M., K. Tanno, A. Kamo, Y. Takayanagi and K. Sasaki: Enhancement of tumor growth by morphine and its possible mechanism in mice. *Biol Pharm Bull* 16, 762-766 (1993)

70. Page, G. G., S. Ben Eliyahu, R. Yirmiya and J. C. Liebeskind: Morphine attenuates surgery-induced enhancement of metastatic colonization in rats. *Pain* 54,

21-28 (1993)

71. Page, G. G., S. Ben Eliyahu and J. C. Liebeskind: The role of LGL/NK cells in surgery-induced promotion of metastasis and its attenuation by morphine. *Brain Behav Immun* 8, 241-250 (1994)

72. Page, G. G., J. S. McDonald and S. Ben Eliyahu: Pre-operative versus postoperative administration of morphine: impact on the neuroendocrine, behavioural, and metastatic-enhancing effects of surgery. *Br J Anaesth* 81, 216-223 (1998)

73. Yeager, M. P. and T. A. Colacchio: Effect of morphine on growth of metastatic colon cancer *in vivo*. *Arch Surg* 126, 454-456 (1991)

74. Hatzoglou, A., E. Bakogeorgou and E. Castanas: The antiproliferative effect of opioid receptor agonists on the T47D human breast cancer cell line, is partially mediated through opioid receptors. *Eur J Pharmacol* 296, 199-207 (1996)

75. Hatzoglou, A., L. Ouafik, E. Bakogeorgou, K. Thermos and E. Castanas: Morphine cross-reacts with somatostatin receptor SSTR2 in the T47D human breast cancer cell line and decreases cell growth. *Cancer Res* 55, 5632-5636 (1995)

76. Bogden, A. E., J. E. Taylor, J. P. Moreau, D. H. Coy and D. J. LePage: Response of human lung tumor xenografts to treatment with a somatostatin analogue (Somatuline). *Cancer Res* 50, 4360-4365 (1990)

77. Prevost, G., E. Foehrle, F. Thomas, I. Pihan, N. Veber, A. Starzec and L. Israel: Growth of human breast cancer cell lines is inhibited by the somatostatin analog BIM23014. *Endocrinology* 129, 323-329 (1991)

78. Setyono-Han, B., M. S. Henkelman, J. A. Foekens and G. M. Klijn: Direct inhibitory effects of somatostatin (analogues) on the growth of human breast cancer cells. *Cancer Res* 47, 1566-1570 (1987)

79. Maneckjee, R. and J. D. Minna: Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. *Proc Natl Acad Sci USA* 87, 3294-3298 (1990)

80. Zagon, I. S., P. J. McLaughlin, S. R. Goodman and R. E. Rhodes: Opioid receptors and endogenous opioids in diverse human and animal cancers. *J Natl Cancer Inst* 79, 1059-1065 (1987)

81. Gupta, K., S. Kshirsagar, L. Chang, R. Schwartz, P. Y. Law, D. Yee and R. P. Hebbel: Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. *Cancer Res* 62, 4491-4498 (2002)

82. Stefano, G. B., M. K. Leung, T. V. Bilfinger and B. Scharrer: Effect of prolonged exposure to morphine on responsiveness of human and invertebrate immunocytes to

- stimulatory molecules. *J Neuroimmunol* 63, 175-181 (1995)
83. Magazine, H. I., J. Chang, Y. Goumon and G. B. Stefano: Rebound from nitric oxide inhibition triggers enhanced monocyte activation and chemotaxis. *J Immunol* 165, 102-107 (2000)
84. Maneckjee, R. and J. D. Minna: Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. *Cell Growth Differ* 5, 1033-1040 (1994)
85. Sueoka, N., E. Sueoka, S. Okabe and H. Fujiki: Anti-cancer effects of morphine through inhibition of tumour necrosis factor- $\alpha$  release and mRNA expression. *Carcinogenesis* 17, 2337-2341 (1996)
86. Shakhov, A. N., M. A. Collart, P. Vassalli, S. A. Nedospasov and C. V. Jongeneel: Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor  $\alpha$  gene in primary macrophages. *J Exp Med* 171, 35-47 (1990)
87. Sueoka, E., N. Sueoka, Y. Kai, M. Suganuma, K. Kanematsu, T. Yamamoto and H. Fujiki: Anticancer activity of morphine and its synthetic derivative, KT-90, mediated through apoptosis and inhibition of NF- $\kappa$ B activation. *Biochem Biophys Res Commun* 252, 566-570 (1998)
88. Jeremias, I., C. Kupatt, B. Baumann, I. Herr, T. Wirth and K. M. Debatin: Inhibition of nuclear factor  $\kappa$ B activation attenuates apoptosis resistance in lymphoid cells. *Blood* 91, 4624-4631 (1998)
89. Welters, I. D., C. Fimiani, T. V. Bilfinger and G. B. Stefano: NF- $\kappa$ B, nitric oxide and opiate signaling. *Medical Hypotheses* 54, 263-268 (1999)
90. Welters, I. D., A. Menzebach, Y. Goumon, P. Cadet, T. Menges, T. K. Hughes, G. Hempelmann and G. B. Stefano: Morphine inhibits NF- $\kappa$ B nuclear binding in human neutrophils and monocytes by a nitric oxide dependent mechanism. *Anesthesiol* 92, 1677-1684 (2000)
91. Provinciali, M., G. Di Stefano, W. Raffaeli, G. Pari, F. Desiderio and N. Fabris: Evaluation of NK and LAK cell activities in neoplastic patients during treatment with morphine. *Int J Neurosci* 59, 127-133 (1991)
92. Barg, J., M. M. Belcheva and C. J. Coscia: Evidence for the implication of phosphoinositol signal transduction in mu-opioid inhibition of DNA synthesis. *J Neurochem* 59, 1145-1152 (1992)
93. Bartolome, J. V., M. B. Bartolome, B. A. Lorber, S. J. Dileo and S. M. Schanberg: Effects of central administration of beta-endorphin on brain and liver DNA synthesis in preweanling rats. *Neuroscience* 40, 289-294 (1991)
94. Kornblum, H. I., S. E. Loughlin and F. M. Leslie: Effects of morphine on DNA synthesis in neonatal rat brain. *Brain Res* 428, 45-52 (1987)
95. Steine-Martin, A. and K. F. Hauser: Opioid-dependent growth of glial cultures: Suppression of astrocyte DNA synthesis by met-enkephalin. *Life Sci* 46, 91-98 (1990)
96. Zagon, I. S. and P. J. McLaughlin: Endogenous opioid systems regulate cell proliferation in the developing rat brain. *Brain Res* 412, 68-72 (1987)
97. Barg, J., M. M. Belcheva, R. Levy, R. J. McHale, J. A. McLachlan, F. E. Johnson, C. J. Coscia and Z. Vogel: A monoclonal anti-idiotypic antibody to opioid receptors labels desipramine-induced opioid binding sites on rat C6 glioma cells and attenuates thymidine incorporation into DNA. *Glia* 10, 10-15 (1994)
98. Barg, J., M. M. Belcheva, R. Zimlichman, R. Levy, D. Saya, R. J. McHale, F. E. Johnson, C. J. Coscia and Z. Vogel: Opioids inhibit endothelin-mediated DNA synthesis, phosphoinositide turnover, and  $\text{Ca}^{2+}$  mobilization in rat C6 glioma cells. *J Neurosci* 14, 5858-5864 (1994)
99. Barg, J., M. M. Belcheva, J. Rowinski and C. J. Coscia: kappa-Opioid agonist modulation of [ $^3\text{H}$ ]thymidine incorporation into DNA: evidence for the involvement of pertussis toxin-sensitive G protein-coupled phosphoinositide turnover. *J Neurochem* 60, 1505-1511 (1993)
100. Mangoura, D. and G. Dawson: Chronic opioid treatment attenuates carbachol-mediated polyphosphoinositide hydrolysis in chick embryo neuronal cultures. *Brain Res* 548, 273-278 (1991)
101. Periyasamy, S. and W. Hoss: Inhibition of carbachol-stimulated phosphoinositide turnover by U-50,488H in rat hippocampus--involvement of GTP-binding protein. *Eur J Pharmacol* 207, 101-109 (1991)
102. Magazine, H. I., Y. Liu, T. V. Bilfinger, G. L. Fricchione and G. B. Stefano: Morphine-induced conformational changes in human monocytes, granulocytes and endothelial cells and in invertebrate immunocytes and microglia are mediated by nitric oxide. *J Immunol* 156, 4845-4850 (1996)
103. Stefano, G. B., A. Hartman, T. V. Bilfinger, H. I. Magazine, Y. Liu, F. Casares and M. S. Goligorsky: Presence of the  $\mu_3$  opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. *Journal of Biological Chemistry* 270, 30290-30293 (1995)
104. Fimiani, C., E. Arcuri, A. Santoni, C. Rialas, T. V. Bilfinger, D. Peter, B. Salzet and G. B. Stefano:  $\mu_3$  opiate receptor expression in lung and lung carcinoma: Ligand binding and coupling to nitric oxide release. *Cancer Letters* 146, 45-51 (1999)
105. Welters, I. D., A. Menzebach, Y. Goumon, T. W.

Langefeld, H. Teschemacher, G. Hempelmann and G. B. Stefano: Morphine suppresses complement receptor expression, phagocytosis, and respiratory burst in neutrophils by a nitric oxide and mu3 opiate receptor-dependent mechanism. *J Neuroimmunol* 111, 139-145 (2000)

106. Nicotera, P., E. Bonfoco and B. Brune: Mechanisms for nitric oxide-induced cell death: Involvement of apoptosis. *Adv Neuroimmunol* 5, 411-420 (1995)

107. Xie, K., S. Huang, Z. Dong, M. Gutman and I. J. Fidler: Direct correlation between expression of endogenous inducible nitric oxide synthase and regression of M5076 reticulum cell sarcoma hepatic metastases in mice treated with liposomes containing lipopeptide CGP 31362. *Cancer Res* 55, 3123-3131 (1995)

108. Xie, K., S. Huang, Z. Dong, S. H. Juang, M. Gutman, Q. W. Xie, C. Nathan and I. J. Fidler: Transfection with the inducible nitric oxide synthase gene suppresses tumorigenicity and abrogates metastasis by K-1735 murine melanoma cells. *J Exp Med* 181, 1333-1343 (1995)

109. Lejeune, P., P. Lagadec, N. Onier, D. Pinard, H. Ohshima and J. F. Jeannin: Nitric oxide involvement in tumor-induced immunosuppression. *J Immunol* 152, 5077-5083 (1994)

110. Wink, D. A., Y. Vodovotz, J. Laval, F. Laval, M. W. Dewhirst and J. B. Mitchell: The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* 19, 711-721 (1998)

111. Kampa, M., A. Hatzoglou, G. Notas, M. Niniraki, E. Kouroumalis and E. Castanas: Opioids are non-competitive inhibitors of nitric oxide synthase in T47D human breast cancer cells. *Cell Death Differ* 8, 943-952 (2001)

112. Stefano, G. B., M. Salzet, C. Rialas, D. W. Mattocks, C. Fimiani and T. V. Bilfinger: Macrophage behavior associated with acute and chronic exposure to HIV GP120, morphine and anandamide: Endothelial implications. *Int J Cardiol* 64, S3-S13 (1998)

113. Gobert, A. P., D. J. McGee, M. Akhtar, G. L. Mendz, J. C. Newton, Y. Cheng, H. L. Mobley and K. T. Wilson: *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: A strategy for bacterial survival. *Proc Natl Acad Sci USA* 98, 13844-13849 (2001)

114. Buga, G. M., L. H. Wei, P. M. Bauer, J. M. Fukuto and L. J. Ignarro: N-omega-hydroxy-L-arginine and nitric oxide inhibit Caco-2 tumor cell proliferation by distinct mechanisms. *Am J Physiol* 275, R1256-R1264 (1998)

115. Singh, R., S. Pervin, A. Karimi, S. Cederbaum and G. Chaudhuri: Arginase activity in human breast cancer cell lines: N(omega)-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. *Cancer Res* 60, 3305-3312 (2000)

116. Chang, C. I., J. C. Liao and L. Kuo: Macrophage

arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Res* 61, 1100-1106 (2001)

**Abbreviations:** cGMP: cyclic guanosine monophosphate; CTOP: D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH<sub>2</sub>; DPDPE: [D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]enkephalin; LAK: lymphokine activated killer; LGL: large granular lymphocytes; NK: natural killer; IkBa: inhibitory kappa B alpha; NFkB: nuclear factor kappa B; NO: nitric oxide; NOS: nitric oxide synthase; cNOS: constitutive nitric oxide synthase; iNOS: inducible nitric oxide synthase; THP: tetrahydropapoverine; TNF: tumor necrosis factor; ODC: ornithine decarboxylase; NOHA: N-omega-hydroxy-L-arginine

**Key Words:** Nitric oxide, Morphine, Opiate receptor, Mu<sub>3</sub> opiate receptor, Neoplasia, Cancer, Apoptosis, Review

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