The \( \mu \)-Opioid Receptor in Cancer Progression

Is There a Direct Effect?

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HERE has been increasing interest in the role of anesthetic drugs and opioids in cancer recurrence and metastasis. Epidemiologic studies in this area have suggested that patients who receive general anesthesia with opioids rather than local or regional anesthetics have a greater rate of cancer recurrence.1 Several retrospective studies have shown a reduced incidence of cancer recurrence after regional anesthesia (epidural, intrathecal, paravertebral) and reduced doses of opioids after surgery for breast, prostate, and colon cancer or melanoma,2–4 although other groups have reported less robust association.5–8 Retrospective studies of cancer or melanoma,2–4 although other groups have reported less robust association.5–8 Retrospective studies of anesthetic choice and disease-free survival studies are subject to confounding factors; however, prospective trials are under way. Assuming the retrospective epidemiologic data demonstrating an effect of anesthetic technique on cancer recurrence are substantiated, it would be important to determine the mechanisms involved. The epidemiologic research has focused largely on potential beneficial effects of regional anesthetics, yet the differences in cancer recurrence rate may be the result of direct or indirect effects on tumor cell growth and metastasis by anesthetic or opioids. Direct effects include stimulating tumor cell proliferation and invasion and inhibiting apoptosis; indirect effects include immunosuppression. The purpose of this review is to assess the possible role of opioids in tumor progression, with a focus on the \( \mu \)-opioid receptor (MOR) and its direct influence on cancer progression.

Indirect Effects of \( \mu \)-Opioids on Cancer Progression

Although this review focuses on evidence of a direct effect of opioids, there is considerable evidence that indirect immunosuppression by opioids could be relevant. One widely studied hypothesis is that various anesthetic drugs or techniques suppress natural killer (NK) cell activity or interfere with the immune response. NK cells, the primary line of defense against tumor cells, can spontaneously recognize and lyse tumor cells. Several reviews on the effects of various anesthetic drugs or techniques on NK cells and their potential role in cancer metastasis have been published.9,10 General anesthesia is reported to decrease concentrations of circulating NK cells, and opioids have a dose-dependent effect on NK cell cytotoxicity.10 Opioid analgesia may also affect other aspects of the immune response: morphine inhibits production of proinflammatory cytokines by monocytes and inhibits interleukin-2 transcription in activated T lymphocytes.11 Clinical studies on immune suppression by opioids during surgery are complex because pain itself may suppress immunity. Distinguishing the immunosuppressive effects of opioids from their analgesic effects has been problematic. Evidence of opioid-induced immunosuppression is clear, but there is a dearth of in vivo evidence that tumor progression is influenced by this mechanism. Nonetheless, despite the current lack of clinical evidence, the use of regional anesthetics, when possible, to minimize immune suppression has been suggested.1,7,9,10,12,13

Direct Effects of \( \mu \)-Opioids on Tumor Progression

There is evidence of a direct effect of \( \mu \)-opioids on tumor progression or recurrence.7,11,14 Opioid receptors are di-
vided into three major subgroups: \( \mu, \kappa \) and \( \delta \). The MOR is the main target for opiates such as morphine, fentanyl, and heroin, with a binding affinity two orders of magnitude greater than the affinity of other opioid receptors. The MOR mediates the main clinical effects of opioids, including analgesia, but also mediates secondary effects such as addiction, respiratory depression, and constipation. Our interest in a direct effect of opioids on tumor progression emerged during the development of the peripheral opioid antagonist methylnaltrexone for opioid-induced constipation. Because methylnaltrexone does not cross the blood-brain barrier, centrally mediated analgesia is preserved. During compassionate use trials in patients with advanced cancer at the University of Chicago, we observed several instances of longer-than-anticipated patient survival, leading us to wonder whether the effect could be attributed to improved gut function or a change in tumor progression.

**Effect of \( \mu \)-Opioids on Angiogenesis**

An important early study by Gupta et al. demonstrated proangiogenic effects of morphine in breast cancer xenografts, when breast cancer cells (MCF-7) were implanted in nude mice. This group found that clinically relevant doses of morphine (10 \( \mu M \)) led to significantly increased tumor volumes \( (P < 0.05) \) and increased tumor vascularization (microvessel density \( P < 0.002 \), total vessel length and branching \( P < 0.001 \)). This effect was inhibited by coadministration of the tertiary MOR antagonist naloxone. Mechanistically, this group demonstrated that morphine promoted activation of the vascular endothelial growth factor (VEGF) receptor 2 and signal transducer and activator of transcription 3 in mouse retinal endothelial cells. This was one of the first studies to demonstrate an effect in vivo on tumor growth and angiogenesis. Morphine (1–100 nM) has also been shown to accelerate keratinocyte migration and wound closure during in vitro chemotaxis and scratch assay models.

In laboratory studies of human dermal and pulmonary endothelial cells, we showed that opioids affect angiogenesis. Both morphine, in clinically relevant concentrations, and \([\text{D-Ala}^2 \text{N-Me-Phe}^4, \text{Gly}^5\text{-ol}]-\text{enkephalin} \) (DAMGO), an experimental synthetic MOR agonist, stimulate endothelial cell migration and proliferation (hallmarks of angiogenesis) by reciprocal transactivation of the VEGF receptor, a well-recognized therapeutic target in antiangiogenic cancer chemotherapy. This effect could be blocked by clinically relevant concentrations of the peripheral \( \mu \)-opioid receptor antagonist, methylnaltrexone, at clinically relevant concentrations. A direct effect of opioids on endothelial cell proliferation was also shown by Leo et al. in a study using human arterial endothelial cells. Morphine \( (0.01–10 \mu M) \) stimulated an increase in endothelial cell proliferation similar to that by VEGF.

**Possible Mechanisms for a Direct Effect of \( \mu \)-Opioids on Cancer Progression**

To further examine the cellular and molecular mechanism of the opioid effect on angiogenesis, we demonstrated that both VEGF and morphine inhibits Src activation, Src-mediated Akt, RhoA, and mammalian target of rapamycin (mTOR) activation and consequent endothelial cell proliferation, migration, and actin cytoskeletal reorganization required for angiogenesis. Methylnaltrexone (MNTX) inhibits the \( \mu \)-opioid receptor (MOR) and promotes tyrosine phosphatase activity, leading to Src inactivation. This promotes Akt, RhoA, and mTOR inactivation and consequent inhibition of angiogenesis.

![Fig. 1. Schematic diagram representing the mechanism(s) by which methylnaltrexone inhibits angiogenesis. Vascular endothelial growth factor (VEGF) binding to VEGF receptors induces Src activation, Src-mediated Akt, RhoA, and mammalian target of rapamycin (mTOR) activation and consequent endothelial cell proliferation, migration, and actin cytoskeletal reorganization required for angiogenesis. Methylnaltrexone (MNTX) inhibits the \( \mu \)-opioid receptor (MOR) and promotes tyrosine phosphatase activity, leading to Src inactivation. This promotes Akt, RhoA, and mTOR inactivation and consequent inhibition of angiogenesis.](https://example.com/fig1.png)
Role of Endogenous Opioids on Cancer Progression

In addition to the effects of *exogenous* opioids, there is substantial laboratory and animal evidence that *endogenous* opioids may play an important role in tumor growth and metastasis. Endogenous opioids such as endorphin and endomorphins are known to be increased after stress (including surgical stress) and may contribute to tumor growth directly or indirectly. In addition to the breast cancer studies by Gupta et al., laboratory studies have demonstrated a link between opioid receptor activation and altered angiogenesis and tumor growth in melanoma, lung cancer, and human squamous cell carcinoma.18,25 Boehncke and tumor growth in melanoma, lung cancer, and human squamous cell carcinoma.18,25 Boehncke et al. reported that endogenous opioids (including those produced by tumor cells) may regulate melanoma growth.25 They demonstrated, albeit in a limited number of patients, a positive correlation between β-endorphin expression and tumor progression. Dai et al., have also published *in vitro* and *in vivo* studies showing that endogenous opioids (endomorphin-1, -2 10 μM) can stimulate angiogenesis in a chick chorioallantoic membrane assay and endothelial cell proliferation, migration, and adhesion *in vitro*.26 These effects were inhibited by naltrexone, a MOR antagonist. Higher doses of the opioids (100 μM) significantly inhibited cell proliferation and migration below baseline levels, indicating that supraphysiologic concentrations of opioids may be cytotoxic. Observations of an effect of the MOR on cancer progression suggest that endogenous opioids have an important biologic role aside from analgesia. This is consistent with previously determined effects of opioids on bacterial and viral function.17

We recently reported four lines of evidence for MOR regulation of cancer progression in animal models independent of exogenous opiates. First, MOR expression is increased more than 5-fold in nonsmall cell lung cancer cell lines and tumor samples. Second, lung cancer cells (Lewis lung carcinoma) do not form tumors when injected into MOR knockout mice.27 Third, silencing of MOR in lung cancer cells transfected with plasmids encoding short-hairpin RNA to achieve a stable knockdown significantly reduces tumor growth and metastasis *in vivo*. Silencing MOR also blocked colony formation, whereas morphine stimulated proliferation of Lewis lung cancer cells *in vitro*. Finally, these results led us to investigate the effects of opioid receptor antagonists in our lung cancer tumor model.27 In animals inoculated with Lewis lung cancer cells and implanted with pumps, both naltrexone and methylnaltrexone significantly inhibited the growth and metastasis of the cancer. Recently, we demonstrated that MOR overexpression promotes growth and metastasis in tumor xenografts in nude mice, suggesting that T-cell immunity is not involved in this process. These results indicate that MOR can directly regulate cancer growth and metastasis in the absence of exogenous opioids such as morphine.28

Although our studies focused on methylnaltrexone, because it can be coadministered with opiates without reversing analgesia and is often used clinically in patients with advanced cancer, the effects of opioid antagonism on cancer progression appear to extend more generally to the class of antagonists. McLaughlin et al., reported that low-dose naltrexone (0.1 mg/kg), administered daily, inhibited tumor growth in a nude mouse model of human squamous cell carcinoma by as much as 84% (P < 0.001).29 Naltrexone has also been tested in a small number of patients as a possible adjuvant therapy along with α-lipoic acid for pancreatic cancer.30 Two clinical trials are under way to assess the effectiveness of naltrexone as a treatment in metastatic breast cancer (NCT00379197) and gliomas (NCT01303835).§

Our data and those from several other laboratories suggest a proangiogenic effect of opioids, but the evidence for opioid-induced angiogenesis is not uniform. Some studies have suggested that opioids may be antiangiogenic and antiproliferative and may trigger apoptosis in cancer cells.11,14,31 These differences may stem from the supraphysiological opioid concentrations used *in vitro* and *in vivo* and the choices of model system (nude mice, cell lines, and such) used.

Human Studies

Although much of the evidence in favor of a proangiogenic effect of opioids is cellular, there is some epidemiologic and genetic support of this hypothesis. One study in palliative care patients showed that patients receiving intrathecal opioids exhibited increased survival (54%) compared with those receiving comprehensive medical management with systemic opioids (37%).32 Although the reasons for longer survival may have included decreased toxicity and better pain relief, one other explanation is that by avoiding systemic opioids and thus tumor exposure to exogenous opioids, cancer progression was attenuated. Because many cancer patients require opioid analgesia, it has been virtually impossible to assess the clinical effects of opioids on tumor progression before the introduction of peripheral opioid antagonists into clinical practice.

Detailed knowledge of the molecular biology of the MOR has led to a recent study suggesting that a common polymorphism may be linked to survival in breast cancer. The MOR is expressed in both the central nervous system and peripheral tissues (fig. 2). The human MOR gene *OPRM1* is 236 kb and consists of 11 exons that give rise to at least 17 splice variants.11,16 MOR-1 is the most abundant transcript and consists of exons 1, 2, 3, and 4 and gives rise to a protein with a molecular weight of approximately 44 kDa. The opioid receptors are members of the seven-transmembrane-spanning, G-protein-coupled receptor superfamily.16 Acute opioid activation can lead to inhibition of adenylate cyclase activity and activation of mitogen-activated protein kinase, phosphatidylinositol 3-kinase (PI3K) and phospholipase C. Desensitization of opioid signaling can occur via receptor endocytosis.33

The most common polymorphism of the MOR receptor A118G results in decreased responsiveness to µ-
opioids. Although it has been evaluated in nicotine addiction, a recent study suggests it may play a role in breast cancer survival. In a study of 2,039 women based on the Carolina Breast Cancer Study, 5% of African Americans and 24% of European American women had one or two copies of the G allele. Of the women with the A/A genotype and invasive cancer, 17% (291 of 1,682) died of the disease versus 8% (27 of 345) of the women with the G allele (P<0.001). It is unclear whether these patients received exogenous opioids, but this survival difference may have been influenced by endogenous opioids.

In a retrospective study of 700 patients with addiction, we assessed the development of new tumors in patients receiving either methadone or implanted naltrexone. We were unable to detect a significant difference in the development of new tumors. However, this study (4,000 patient years) was not designed or statistically powered to assess recurrence rates in patients with preexisting malignancies.

The perioperative period is recognized as a unique and stressful period for the patient. First, μ-opioid agonists are routinely given parenterally during the perioperative period and often in high doses. Second, in cases of tumor surgery, recent data suggest that tumor cells may be released into the circulation. Studies have shown a correlation between disseminated tumor cells and metastatic relapse. Finally, opioids may affect endothelial barrier function directly so that tumor cells liberated during surgery may directly breach the barrier and invade the underlying tissues, potentially causing metastasis. The endothelial barrier regulates many vascular functions, including vascular permeability, blood pressure, and immune cell infiltration. A decrease in barrier integrity is observed during angiogenesis. Tumor vasculature is often leaky and disorganized, facilitating tumor cell extravasation. We previously demonstrated a concentration-dependent change in barrier integrity in human pulmonary endothelial cells exposed to morphine or other MOR agonists in clinically relevant concentrations. Methylnaltrexone attenuated the endothelial barrier disruption produced by morphine or [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO) in vitro and reduced lipopolysaccharide-induced vascular hyperpermeability in the murine lung in vivo. Endothelial barrier disruption, migration, and proliferation are all necessary steps for angiogenesis.

**Conclusion**

Despite the evidence from cellular and epidemiologic animal studies, clinicians should note that there are no controlled trials in humans demonstrating a direct effect of opioids in facilitating tumor progression or of opioid antagonists in attenuating tumor progression. Animal models do not uniformly translate into human disease. In addition, certain caveats apply to our work. Lewis lung carcinoma is among the most robust tumors in both growth and response to drugs. Whether our findings are applicable to other tumors or to humans is not known. In addition, the work presented in this communication deals with the effects of MOR antagonists, and the effect of chronic opioid administration has not been specifically assessed. There is considerable evidence that chronic exposure to exogenous opioids changes the response in both brain and gut. Whether MOR expression changes with chronic exposure in tumor cells or endothelial cells is unknown. Finally, although there may be evidence of a direct effect of opioids, these studies do not preclude other effects of opioids, either directly or indirectly on immune function. The clinical trials currently under way using different anesthetic and analgesic techniques in cancer surgery should provide important insights into the role of opioids in tumor progression.
surgery will provide additional information on the effect of opioids on cancer progression and recurrence and also on immune cell function. Outcome measures for the studies include local and metastatic cancer recurrence, NK and other immune cell functions, and disease-free survival. The development of peripherally restricted \(\mu\)-opioid antagonists may permit laboratory and clinical assessment of whether there is a meaningful relationship between the MOR and cancer progression.\(^{23}\) In summary, there are epidemiologic, animal, and cellular studies that suggest a possible therapeutic role of MOR antagonists on cancer growth and metastasis that merit additional research.

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ANESTHESIOLOGY REFLECTIONS

The Faraday Company “Distention Attachment” for “Oxygen Cure” of Piles

Before many anesthesiologists and other physicians became involved in respiratory therapy, there were quacks marketing “oxygen cures.” Not long after federal laws in 1906 forced disclosure of ingredients upon nostrum manufacturers, charlatans like the Faraday Company of New Haven, Connecticut began selling ersatz apparatus in a booklet (left) promoting, among other things, “cures” for hemorrhoids: Oxygenopathy: A Message of Hope to All Suffering and Afflicted Ones. Wired to a piece of plumbing, the Connecticut firm’s “Distention Attachment” (right) was supposed to be anally inserted for 20- to 30-minute sessions at least three to four times daily, until a patient with piles found relief from constipation. According to oxygenopaths, this constipation cure would in turn improve “Asthma, Bronchitis, Liver and Stomach Disorders,” palpitations, “Neurasthenia, Headache, Spinal Irritation, Insomnia, Female Complaints, Paralysis, Neuralgia, Vertigo, Hysteria, Epilepsy, Dyspepsia, etc. . .” (Copyright © the American Society of Anesthesiologists, Inc.)

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