Morphine and tumor growth and metastasis

Banafsheh Afsharimani · Peter Cabot · Marie-Odile Parat

Abstract Morphine is an analgesic widely used to alleviate cancer pain. In addition, the perioperative management of pain in cancer surgery patients most often includes opioids. However, there are reports that these drugs may alter cancer recurrence or metastasis. Several mechanisms have been proposed, such as the modulation of the immune response or cellular pathways that control the survival and migratory behavior of cancer cells. The published literature, however, presents some discrepancies, with reports suggesting that opioids may either promote or prevent the spread of cancer. It is of great importance to determine whether opioids, in particular the most widely used, morphine, may increase the risk of metastasis when used in cancer surgery. This review examines the available data on the effects of morphine which influence cancer metastasis or recurrence, including immunomodulation, tumor cell aggressiveness, and angiogenesis, with special emphasis on recently published clinical and laboratory based studies. We further discuss the parameters that may explain the difference between reports on the effects of morphine on cancer.

Keywords Morphine · Opioids · Invasion · Metastasis

1 Introduction

Opioids are widely used in the pain management of cancer patients, and not surprisingly, interest in the possibility that these may alter the course of cancer is not recent [1]. However, there has been recent awareness regarding the use of opioids in cancer surgery patients based on a combination of (1) multiple articles from the 1990s indicating that morphine has immunosuppressant properties which can promote cancer, but that suppressing pain alleviates the surgical stress and thus might be protective against tumor metastasis; (2) more recent cellular and biological in vitro and in vivo studies exploring pathways by which opioids may directly or indirectly promote or prevent cancer, including recent literature on morphine and angiogenesis; and (3) recent retrospective clinical studies and ongoing prospective trials testing whether regional anesthesia and analgesia offers protection against metastasis or recurrence when compared to general anesthesia with opioid analgesia. This review attempts to clarify the positive and negative effects of opioids, more specifically morphine, on tumor growth and metastasis which are mediated by a combination of direct effects on tumor cell proliferation and invasion and indirect effects on processes that are key to tumor development, such as immunity, inflammation, or angiogenesis.

2 Direct effect of morphine on tumor cells

2.1 Opioids affect tumor cell proliferation and apoptosis

The three major types of receptors to which endogenous and exogenous opioids bind—the \( \mu \)-, \( \kappa \)-, and \( \delta \)-opioid receptors—have been identified not only in peripheral...
sensory neurons and the CNS but also in various other cell types and, in cancer cells, via detection of the corresponding mRNA [2], the protein [3, 4], or the aptitude of the cells to bind [5–8] and/or respond to opioids. Opioids directly regulate the growth of normal and neoplastic cells by modulating cell proliferation and/or apoptosis, which gives these drugs the theoretical capability to regulate tumor growth and metastasis. However, reports are discrepant, depending on the cell type studied and the dose of morphine or other opioid used. Whether direct effects of morphine on tumor cells matter in vivo and whether the final outcome of morphine administration to a patient would be the inhibition or promotion of malignant cell proliferation and survival is still debatable.

Several reports have documented a pro-apoptotic effect of opioids on cancer cells in vitro at nanomolar [6], micromolar [9], or millimolar [10, 11] concentrations either by quantifying apoptotic cells [12] or by measuring hallmarks of apoptosis such as the cleavage of pro-apoptotic caspases or the release of cytochrome c from the mitochondria [10, 11]. Pathways proposed to be involved in the effect of opioids on apoptosis include the activation of the anti-apoptotic kinase Akt, activation of c-Jun N-terminal kinase, generation of reactive oxygen species, generation of nitric oxide, increased expression of pro-apoptotic Bim, and decreased expression of anti-apoptotic Bel-2, as well as p53- and NFκB-mediated pathways [9–11, 13]. P53 is a key player in the regulation of apoptosis, and it has been shown to be involved in morphine-induced apoptosis via (1) morphine-induced stabilization of p53 and (2) morphine-induced apoptosis in cells with normal p53, but lack of an effect in cells with mutant dominant-negative p53 [14]. NFκB is a transcription factor which, besides controlling the immune response to stress, regulates several genes associated with apoptosis and angiogenesis in various tissues. It is suggested that morphine can block NFκB directly by modulation of NO release [13].

The involvement of the opioid receptor(s) in the anti-proliferative effect of morphine is not always clear, as shown by the reports assembled in Table 1. At nanomolar concentrations, morphine and other opioid agonists were shown to inhibit the proliferation of a breast cancer cell line that had κ- and δ-opioid but not μ-opioid receptors, and the authors hypothesized that morphine interacted with receptor systems other than opioid receptors [6]. The somatostatin receptor system has been proposed as a potential candidate, and the interaction of opioid signaling with nicotine or estrogen signaling in the regulation of the proliferation of tumor cells has also been suggested [5, 15, 16]. An effect of opioids on the cytoskeleton could also mediate their antiproliferative action [17]. Morphine has been shown to possess anticancer effects at high doses (1–10 mM) by inhibiting the growth of several cancer cell lines in vitro and inhibiting TNF-α expression [18, 19]. Nitric oxide or reactive oxygen species (ROS) have direct and indirect tumoricidal effects, and morphine stimulates their production and release, which could also account for some of the anti-tumor effects of morphine [13].

In contrast, the anti-apoptotic actions of morphine in tumor cells subjected to pro-apoptotic stimuli have been reported [20, 21]. Similarly, morphine-induced increased proliferation was documented in K562 leukemia cells [22]. It is likely that this variability could be accounted for by cell-specific activity. However, the majority of published reports point to the ability of exogenous opioids to prevent rather than promote the survival and proliferation of tumor cells.

2.2 Opioids alter tumor cell invasion

Tumor cell invasion requires cellular processes such as adhesion, extracellular matrix degradation, and cell migration. Studies have indicated that opioids can modulate all of these processes. The adhesion of cells to the extracellular matrix components collagen type I and fibronectin was reduced by micromolar concentrations of morphine, and so was adhesion to type IV collagen, while adhesion to laminin or basement membrane was unaltered. The cells used in this study were non-cancer cells engineered to express functional μ-opioid receptor. Mechanistic insight indicated reduced phosphorylation of kinases involved in focal adhesions [23]. Adhesion of colon cancer cells to collagen type IV was reduced as well in cells pretreated with morphine in another study [24].

The migratory behavior of different cell types responds differently to morphine: decreased for immune cells in a number of studies as described in our immune suppression paragraph, decreased or increased for endothelial cells as described in our angiogenesis section, increased for microglial cells [25], decreased in colon carcinoma cells [24], and unchanged in multiple cancer cell lines [26].

Invasion relies on matrix digestion by enzymes secreted by the cancer cells themselves or by stromal cells in response to cancer cells. Matrix metalloproteases and urokinase type plasminogen activator are known to play a key role in this process. Morphine inhibits matrix metalloprotease (MMP)-2 and MMP-9 expression [24, 27] but enhances uPA production [4, 28] by breast and colon cancer cells. Invasion into reconstituted basement membrane (Matrigel) was shown to be decreased in colon cancer cells [24] but unchanged in a number of cancer cell lines tested in another study [26].

Except for the documented increase in uPA, the in vitro data therefore indicate that by acting directly on the tumor
cells, morphine may reduce their invasive potential. It should be noted that the direct effect of morphine on tumor cells may not be as important in promoting or preventing tumor growth and metastasis as the effect of morphine on the stroma, whose involvement in regulating the cancer growth is receiving increasing attention.

3 Indirect effects of morphine on tumor growth and metastasis

3.1 Opioids are immunosuppressive

The vast majority of reports indicate that morphine and other exogenous opioids are immunosuppressive. The immune system plays a key role in the control of tumor formation and metastasis. Tumor cells express non-self-antigens and are subjected to killing by activated T and NK cells and to the cytotoxic action of cytokines such as interferon gamma [29, 30]. Mice lacking various components of the immunosurveillance system are more susceptible to tumorigenesis [29, 30].

Moreover, after cancer cells have escaped immunosurveillance and a tumor develops, innate and adaptive immune cells are also present in the tumor microenvironment where they can promote or fight tumor development [30, 31]. Acute and chronic administration of exogenous opioids including the one most commonly used—morphine—inhibits components of the cellular and humoral immune function such as antibody production, NK cell activity, cytokine expression, blood lymphocyte proliferative responses to mitogen, and phagocytic activity [32–35]. While many studies have tested the immunosuppressive effects of morphine in vitro and in vivo in rodents and humans [32, 33, 35, 36], other opioids have been shown to also possess immunosuppressive effects, e.g., therapeutic agents such as fentanyl [37–39], or pharmacological tools such as subtype-specific opioid receptor agonists U50 488H, deltorphin II, or DPDPE [40].

Opioid receptors have been identified in cells of the immune system such as polymorphonuclear leukocytes, macrophages, T lymphocytes, splenocytes, macrophage-like, and T cell-like cell lines [33]. Opioids added directly to a number of cell types of the immune system in vitro alter their protein expression profile [41] and their function: reduced chemotaxis and phagocytosis by macrophages, reduced B-cell proliferation, and suppression of antibody formation [42]. However, a number of studies have failed to find a direct effect of morphine in vitro on NK cell activity. In addition, in contrast to exogenous opioids, an increase in natural killer cell activity in response to endogenous opioids has been documented [43], which was inhibited by naloxone.

Opioids are now known to modulate the immune response by a combination of central and peripheral mechanisms [44]. Signals of central origin may be relayed through (1) the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the production of glucocorticoids which are immunosuppressive, and (2) the sympathetic nervous system eliciting the release of biologic amines into lymphoid organs, which also reduce immunocompetence [45]. Studies have documented that corticosteroids only partially mediate morphine immunosuppression [46–48]. It has been suggested that the HPA axis is involved in the immunosuppression by chronic opioid exposure, while the sympathetic nervous system mediates the immunosuppression induced by acute opioid administration [49]. A receptor-mediated effect has been proposed based on the finding that morphine immunosuppression is abolished in mice genetically deprived of the μ-opioid receptor [50].

In addition, withdrawal from morphine in dependent mice has also been shown to induce marked immunosuppression [51].

It should be noted that although the immunosuppressive effect of opioids is widely documented, there are a number of studies showing a favorable immunomodulatory effect of perioperative morphine administration on surgical stress effects and metastasis promotion (Table 2), presumably due to the relief of pain and the attenuation of the pain component of surgical stress. Given the immune suppression intrinsic to morphine, alleviating the postoperative pain via other means is proposed as an alternative to offer better protection than morphine against surgical stress-induced immune suppression and tumor promotion. Using alternate opioids such as tramadol or the partial μ-agonist buprenorphine [35, 39] or anti-inflammatory drugs [52–54] has been suggested to be beneficial following findings in either animal or clinical studies. In addition, utilizing regional anesthesia and analgesia is another option that has attracted a lot of recent attention, as discussed later in this review. Results from studies testing the effect of morphine on the immune response and tumor burden are assembled in Table 2.

3.2 Opioids and inflammation are closely interrelated

Inflammation, including its subclinical forms, is thought to mediate the tumorigenic effect of most cancer risk factors, including tobacco, obesity, infections, and old age [30]. Inflammation further modulates the host immune response to tumors [30], and tumor development is accompanied by an inflammatory response which creates a pro-tumorigenic microenvironment [30]. Opioids regulate the expression of inflammatory cytokines and their receptors, and immune cells under the influence of cytokines can release endogenous opioids at sites of inflammation [33, 55, 56]. It has
<table>
<thead>
<tr>
<th>Study model</th>
<th>Morphine dosage and duration of administration</th>
<th>Effect on cell growth and survival</th>
<th>Proposed mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 human breast carcinoma cells</td>
<td>$10^{-8}$–$10^{-7}$ M for 24 h</td>
<td>Decrease in cell proliferation</td>
<td>Activation of μ- and κ-opioid receptors on the tumor cells; reversed by naloxone; dependent on presence of oestrogens</td>
<td>[16]</td>
</tr>
<tr>
<td>Multiple lung cancer cells</td>
<td>$10^{-9}$–$10^{-7}$ M for 24 h</td>
<td>Decrease in cell proliferation</td>
<td>Activation of μ-κ- and δ-opioid receptors on tumor cells Effect of morphine inhibited by nicotine</td>
<td>[5]</td>
</tr>
<tr>
<td>EL-4 leukemia cells, P388, MM-46 and Meth-A cells</td>
<td>350 μM to 2.8 mM for 48/72 h</td>
<td>Inhibition of cell growth with different IC50 values for different cell lines</td>
<td>Increased proliferation of leukemia cells</td>
<td>[98]</td>
</tr>
<tr>
<td>K562 leukemia cells</td>
<td>$10^{-9}$–$10^{-6}$ M for 24 h</td>
<td>Increased apoptosis</td>
<td>Activation of opioids receptors and reduction in PKC activity</td>
<td>[99]</td>
</tr>
<tr>
<td>Lung cancer cells NCI-Hi 57 (non-small cell), NCI-N4i 7 and NCI-H146 (small cell)</td>
<td>$10^{-8}$–$10^{-7}$ M for 24 h</td>
<td>Increased apoptosis</td>
<td>Effect of morphine inhibited by nicotine</td>
<td></td>
</tr>
<tr>
<td>T47D human breast cancer cells</td>
<td>$10^{-12}$–$10^{-6}$ for 4 days</td>
<td>Dose-dependent inhibition of cell proliferation (IC50 = 1.08 nM)</td>
<td>Interaction of morphine with the somatostatinergic system</td>
<td>[15]</td>
</tr>
<tr>
<td>T47D human breast cancer cells</td>
<td>1–100 nM for 5 days</td>
<td>Dose-dependent inhibition of cell proliferation</td>
<td>Effect of morphine independent from μ-opioid receptors</td>
<td>[6]</td>
</tr>
<tr>
<td>Human prostate cancer cells (LNCaP, DU145, and PC3)</td>
<td>$10^{-10}$ to $10^{-9}$ M for 4–5 days</td>
<td>Inhibition of tumor cell proliferation</td>
<td>Activation of κ-opioid receptors or effect of morphine not mediated by opioid binding sites depending on the cell line tested</td>
<td>[7]</td>
</tr>
<tr>
<td>PC9 (lung cancer cells) KATO III (stomach cancer cells) HL-60 promyelocytic leukemia cells Neuroblastoma cells U251 glioma cells SEK1 melanoma cells SKNO-1 leukemia cells</td>
<td>Incubation with high concentrations of morphine (mM range) for 3–4 days</td>
<td>Morphine inhibits tumor cell growth in a dose-dependent manner</td>
<td>Inhibition of NFκB activation; suppression of TNF-α expression</td>
<td>[18, 19]</td>
</tr>
<tr>
<td>Human oral tumor cells (HSC-2 and HSG)</td>
<td>$10^{-5}$–$5 	imes 10^{-4}$ M</td>
<td>Cytotoxicity of morphine</td>
<td>–</td>
<td>[100]</td>
</tr>
<tr>
<td>Colon carcinoma cells 26-L5</td>
<td>0.0–0.35 μM for 24 h</td>
<td>Unchanged cell growth</td>
<td>–</td>
<td>[24]</td>
</tr>
<tr>
<td>MCF-7, MDA-MB231 breast carcinoma cells HT-29 colon cancer cells</td>
<td>$10^{-5}$–$2 	imes 10^{-3}$ M for 24 h</td>
<td>Reduction in tumor cell proliferation at low dose, induction of apoptosis at high doses Lower effect on HT-29 cells</td>
<td>Naloxone-independent p53 stabilization and subsequent increase in pro-apoptotic factors p21, Bax, Fas death receptor</td>
<td>[14]</td>
</tr>
<tr>
<td>SC injection in nude mice of MCF-7, MDA-MB231 breast carcinoma cells HT-29 colon cancer cells</td>
<td>IP 10–30 mg kg⁻¹ day⁻¹ for 3 weeks (resulting in 0.9–60 μM circulating concentration)</td>
<td>Reduction in tumor size for MCF-7, MDA-MB231 breast cancer cells but not HT-29 colon cancer cells</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SH-SY5Y neuroblastoma cells</td>
<td>$10^{-7}$–$10^{-5}$ M for 24 h</td>
<td>Protects against serum withdrawal-induced cell death</td>
<td>μ-opioid receptor activates a Gi/0 linked, PI3Kinase/Akt pathway</td>
<td>[20]</td>
</tr>
<tr>
<td>HT-29 colon cancer, MIA PaCa-2 pancreatic cancer CAL-27 squamous cell carcinoma of the head and neck</td>
<td>$10^{-6}$ M for 2, 5 and 7 days</td>
<td>Increase in tumor cell apoptosis Increase in cell necrosis</td>
<td>Pro-apoptotic effect of morphine partially reversed by naloxone</td>
<td>[12]</td>
</tr>
<tr>
<td>Jurkat cells (leukemia)</td>
<td>$10^{-5}$ or $3 	imes 10^{-5}$ M for 36 h</td>
<td>Increase in apoptosis</td>
<td>Activation of FADD/p53, anti-apoptotic PI3K/Akt and NF-κB pathways Inhibition of ROS and mitochondrial cytochrome c release by morphine Inhibition of NFκB activation</td>
<td>[9] [21]</td>
</tr>
<tr>
<td>Neuroblastoma cells (SH-SY5Y)</td>
<td>Morphine (50–200 μM) was added 1 h before doxorubicin treatment (48 h)</td>
<td>Inhibition of doxorubicin-induced apoptosis</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>
been suggested that the κ- and μ-opioid receptors play opposing roles, with the κ-opioid receptor activation inducing an anti-inflammatory response while the μ-opioid receptor favors a pro-inflammatory response [57]. However, the picture is far more complex. Morphine has been shown to prevent inflammation-induced angiogenesis in vivo [58]. The modulation of inflammation may therefore provide mechanistic insights into how morphine and other opioids influence the development of cancer.

3.3 Morphine modulates angiogenesis

Recent publications point to a role of morphine in angiogenesis (Table 3). Endothelial cells express opioid receptor(s) [59, 60] and respond to opiates by increasing intracellular calcium concentration and nitric oxide production [59–62]. In addition to vascular permeability, nitric oxide regulates endothelial cell proliferation, migration, and protease release, all of which are important for angiogenesis. Clinically relevant concentrations of morphine result in the activation of mitogen-activated protein kinase/ extracellular signal-regulated kinase MAPK/ERK and pro-survival Akt in cultured endothelial cells [60]. In vitro, morphine was shown to induce endothelial cell proliferation and migration via the transactivation of growth factor receptors that play a key role in migration and survival of these cells, including the vascular endothelial growth factor (VEGF) receptor 2 [63, 64] or the platelet-derived growth factor (PDGF)-beta receptor [63]. In a non-endothelial cell system, morphine was further shown to transactivate the receptor (FGFR1) of another growth factor playing a key role in angiogenesis, namely fibroblast growth factor (FGF) [65]. Accordingly, morphine enhanced endothelial cell proliferation and tube formation in vitro and increased in vivo blood vessel formation in a Matrigel plug assay [60] and in tumor xenographs [60, 66]. Morphine further improved wound healing, possibly via an angiogenesis-mediated mechanism [67]. The opioid antagonist methylnaltrexone was shown to prevent VEGF-induced angiogenesis in an in vivo Matrigel plug assay [68]. In vitro pro-angiogenic effects are also reported with physiological concentrations of the endogenous opioids endomorphin-1 and endomorphin-2, and deltorphin 1 [69]. Of note is that some of the pro-angiogenic effects of morphine are not reversed by naloxone [60], but morphine-induced tumor growth and angiogenesis are prevented by co-administration of the cyclooxygenase-2 COX-2 inhibitor celecoxib [70].

One mechanism through which the VEGF pathway increases endothelial cell migration involves adhesion molecules, especially intercellular adhesion molecule 1 or ICAM-1: VEGF stimulation of endothelial cells increases ICAM-1 expression [71], VEGF-induced endothelial cell migration is decreased by a ICAM-1 blocking antibody [71], and ICAM-1-deficient endothelial cells exhibit reduced eNOS activation, NO bioavailability, and migration [72]. ICAM-1 is reported to not only increase endothelial cell migration [72] but also to mediate the recruitment of endothelial progenitor cells, which participate in angiogenesis [73]. Interestingly, endothelial cells exposed to μ-agonists upregulate ICAM-1 [74]. Taken together, these data suggest that morphine transactivation of VEGF receptor 2 may result in an increased endothelial cell motility mediated by nitric oxide and ICAM-1.

Table 1 (continued)

<table>
<thead>
<tr>
<th>Study model</th>
<th>Morphine dosage and duration of administration</th>
<th>Effect on cell growth and survival</th>
<th>Proposed mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer HT-29 cells</td>
<td>Morphine 1 nM to 1 μM for 24 h</td>
<td>No significant change in cell proliferation</td>
<td>–</td>
<td>[4]</td>
</tr>
<tr>
<td>MCF-7 and MDA-MB231 breast cancer cells</td>
<td>500 μM to 2 mM</td>
<td>Increase in tumor cell apoptosis</td>
<td>Decreased activation of Akt, increased cleavage of caspase 8, and inhibition of morphine-induced cell death by β-arrestin</td>
<td>[10]</td>
</tr>
<tr>
<td>MCF7 breast cancer cells</td>
<td>10⁻⁸–10⁻⁴ M morphine for 2, 3, and 5 days</td>
<td>No change in cell numbers at any concentration or time assayed</td>
<td>–</td>
<td>[28]</td>
</tr>
<tr>
<td>Neuroblastoma cells (SH-SY5Y)</td>
<td>0.5–4 mM for 48 h</td>
<td>Dose-dependent increase in apoptosis</td>
<td>Activation of c-Jun N-terminal kinase and increased in reactive oxygen species upregulation of pro-apoptotic protein Bim and downregulation of anti-apoptotic protein Bcl-2 increase in cytochrome c release and caspase-3 and 99 activation</td>
<td>[11]</td>
</tr>
<tr>
<td>Lewis lung carcinoma cells</td>
<td>1 nM for 24 h</td>
<td>Increased proliferation</td>
<td>μ-opioid receptor dependent</td>
<td>[3]</td>
</tr>
<tr>
<td>Study model</td>
<td>Morphine dosage and duration of exposure</td>
<td>Tumor type</td>
<td>Effect on tumor</td>
<td>Immunomodulatory effect</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------</td>
<td>------------</td>
<td>----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>SC injection (20 mg/kg) 1 day before and 2 days after tumor cell inoculation</td>
<td>Colon adenocarcinoma cells injected into cecal vein</td>
<td>Reduced incidence and weight of hepatic metastases</td>
<td>–</td>
</tr>
<tr>
<td>In vivo (mice)</td>
<td>Daily SC injection of 10 mg/kg for 10 days, starting 24 h after tumor cell inoculation</td>
<td>SC growth of solid tumors: EL-4 leukemia cells and Sarcoma 180 carcinoma. IP growth of ascites tumor cells: P388 leukemia and Meth-A fibrosarcoma</td>
<td>Increase in the weight of solid tumors</td>
<td>Decrease in the survival rate of mice</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>SC injection of 15 mg/kg —4 times in the perioperative 24 h</td>
<td>Injection of syngeneic colon adenocarcinoma cells in ileocecal vein + standardized surgical stress</td>
<td>Significant increase in hepatic tumor burden</td>
<td>NK cytotoxicity suppressed</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>IP injection 5 mg/kg 30 min before surgery, SC slow-release 5 mg/kg immediately after surgery and again at time of tumor cell inoculation</td>
<td>Tail vein injection of MADB106 mammary adenocarcinoma cells 5 h after laparotomy</td>
<td>Attenuation of the surgery-induced increase in retention of malignant cells in lungs</td>
<td>–</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>IP injection 10 mg/kg 30 min before surgery, SC slow-release 5 mg/kg immediately after surgery and after tumor cell inoculation</td>
<td>Tail vein injection of MADB106 mammary adenocarcinoma cells 4 h after laparotomy</td>
<td>Attenuation of the surgery-induced increase in retention of malignant cells in lungs</td>
<td>? —The surgery induced decrease in NK cell cytotoxicity is not altered by morphine. However morphine’s protective effects require the presence of NK cells</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>IP injection 8 mg/kg 30 min before surgery and/or SC slow-release 4 mg/kg immediately after surgery and/or 2 mg/kg at the time of tumor cell inoculation</td>
<td>IV injection of MADB106 mammary adenocarcinoma cells 4 h after laparotomy</td>
<td>Attenuation of surgery-induced increase in retention of malignant cells in lungs by all timings of morphine administration. Preoperative morphine the most efficient</td>
<td></td>
</tr>
<tr>
<td>In vitro</td>
<td>0.3–300 ng/ml for 5 days during co-culture or repeated addition of 3 ng/ml to CM for 5 days</td>
<td>T cell leukemia cells (MT-2) induced by HTLV-1 (human leukemia virus)</td>
<td>Enhanced cell death in MT-2 malignant cells exposed to the sensitized cytotoxic T cells treated with morphine</td>
<td>Stimulation of the cytotoxic T cells activity Increase in the number of IFN gamma-producing CD8+ T cells</td>
</tr>
<tr>
<td></td>
<td>3.2 pg/ml to 32 μg/ml for 16 h and daily addition of 3 ng/ml to CM for 5 days</td>
<td>T cell leukemia cells (MT-2) induced by HTLV-1 (human leukemia virus)</td>
<td>No significant difference in the number of dead cells when exposed to 6 different mononuclear cell samples treated or not treated with morphine</td>
<td>No effect on NK activity</td>
</tr>
<tr>
<td>In vivo (rats)</td>
<td>IP injection 10 mg/kg immediately before laparotomy Spinal blockade with</td>
<td>Tail vein injection of MADB106 mammary adenocarcinoma</td>
<td>Systemic morphine attenuated the surgery-induced increase in retention of malignant cells</td>
<td>No significant effect on immune defenses by systemic morphine was measured</td>
</tr>
</tbody>
</table>
In contrast to all the literature reporting the pro-angiogenic effect of morphine, there is a body of evidence indicating that morphine is detrimental to endothelial cells and to neovascularization. An early study showed reduced angiogenesis in the chicken chorioallantoic membrane (CAM) assay in the presence of morphine [75], which was later corroborated by the opioid receptor-dependent reduced angiogenesis shown in the CAM assay using the endogenous opioid [Met⁵]-enkephalin [76]. High concentrations of morphine are toxic to endothelial cells in vitro [62, 77] and result in oxidative stress-mediated endothelium dysfunction in vivo [77]. Accordingly, morphine reduced wound healing and mobilization of endothelial progenitor cells in mice and decreased the formation of capillaries in vitro as well as in an in vivo Matrigel plug assay [58, 78, 79]. In the context of ischemia, where neovascularization is beneficial via the development of collateral circulation, morphine has been shown to prevent hypoxia-induced VEGF production in vitro and in vivo, and thus presumably angiogenesis [80, 81]. These results were confirmed using a mouse tumor angiogenesis assay where the effect of morphine was abolished by the pharmacological or molecular ablation of the μ-opioid receptor [82]. Morphine was also shown to indirectly inhibit angiogenesis in in vivo models via decreased inflammation [58, 79]. Finally, naltrexone alone has been suggested to increase angiogenesis in vivo in two separate models, indicating the potential tonic inhibition exerted by endogenous opioids [76, 83].

At present, whether morphine would impact angiogenesis when administered to cancer patients is still unclear. Several considerations about experimental conditions can explain the discrepancy between results from different groups on the effect of morphine on angiogenesis. In vitro experiments often use endothelial cells of human origin, while in vivo angiogenesis experiments use rodents. Analysis of the opioid receptor(s) present on endothelial cells demonstrated sensitivity to opiate alkaloids but insensitivity to opioid peptides (a profile characteristic of the μ3-opioid receptor) in both rat and human endothelial cells [59]. The concentrations of morphine used in the experiments showing anti-angiogenic properties were reportedly high—up to 100 μM and even 1 mM in vitro and up to doses of 20–30 mg kg⁻¹ day⁻¹ in mice. Concentrations of 1 mM morphine are reportedly toxic to several cell types in vitro. The relatively short half-life of morphine in mice would suggest that studies with intermittent administration of morphine could provide different kinetics of receptor exposure to circulating levels of morphine compared to studies using minipump-controlled release. Furthermore, mice process morphine differently from humans and generate mostly morphine-3-glucuronide, which is inactive on the MOR, whereas humans generate both active M6G and inactive M3G. Justifying a morphine dose administered to mice based on a human relevant dose adjusted for body weight is therefore irrelevant.

### 3.4 Perioperative opioids in tumor surgery patients

While they appear to be an essential component in the pain management of terminal cancer patients, opioids, and in particular morphine, are also used perioperatively in patients undergoing solid tumor resection. In this context, the potential immunosuppressive and pro-angiogenic properties of morphine have raised concerns.

It is well known that in non-cancer surgery patients, regional analgesia prevents the postoperative drop of immune response compared to opioid analgesia. Epidural
### Table 3: Effect of opioids on tumor angiogenesis

<table>
<thead>
<tr>
<th>Type of opioid</th>
<th>Type of study</th>
<th>Morphine dosage and duration of exposure</th>
<th>Study model</th>
<th>Effect on angiogenesis</th>
<th>Proposed mechanism (s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine sulfate</td>
<td><em>In vivo</em></td>
<td>24–96 h 5, 10, 50 μg (peak angiostatic effect (53%) with 5 μg at 72 h)</td>
<td>Chick embryo chorioallantiotic membrane</td>
<td>Angiostatic effects (lower doses (5 μg) caused faster and more intense effect than higher doses (10 μg/50 μg))</td>
<td>Not reversed by Naloxone</td>
<td>[75]</td>
</tr>
<tr>
<td>β-endorphin</td>
<td><em>In vivo</em></td>
<td>24–96 h 5, 10, 15 μg</td>
<td>Chick embryo chorioallantiotic membrane</td>
<td>Time- and dose-dependent angiostatic effect (peak at 10 μg)</td>
<td>Stimulation of IFN production by LGL/NK cells Inhibition of PG-E2 as a pro-angiogenic factor</td>
<td></td>
</tr>
<tr>
<td>[Met5]-enkephalin</td>
<td><em>In vivo</em></td>
<td>5 μg per disk</td>
<td>Chick embryo chorioallantiotic membrane</td>
<td>Decreased number of blood vessels and total vessel length</td>
<td>Action reversed by naloxone; Methylaltrexone alone increases angiogenesis, suggesting tonic inhibition by endogenous opioid(s)</td>
<td>[76]</td>
</tr>
<tr>
<td>Morphine</td>
<td><em>In vitro</em></td>
<td>1.5–150 nM for 24 h</td>
<td>Mouse heart microvascular endothelial cells Human umbilical vein endothelial cells</td>
<td>Increased neovascularization</td>
<td>Morphine elicits NO release, and MAPK/ERK signaling Morphine inhibits EC apoptosis and activates survival promoting Akt The pro-angiogenic effect of morphine is not reversed by naloxone in the Matrigel plug assay but is reversed by naloxone in the tumor angiogenesis assay</td>
<td>[80]</td>
</tr>
<tr>
<td>Morphine; Fentanyl; Hydromorphone</td>
<td><em>In vitro</em></td>
<td>0.1 μM in the migration assay 0.1 μM for 24 h in the proliferation assay</td>
<td>Human dermal microvascular EC</td>
<td>Increased proliferation and migration of endothelial cells</td>
<td>Upregulation of eNOS and iNOS in the wound; upregulation of VEGF receptor Flk1 VEGF receptor transactivation reversed by methylaltrexone</td>
<td></td>
</tr>
<tr>
<td>Morphine; morphine-6-glucuronide; DAMGO</td>
<td><em>In vitro</em></td>
<td>0.01–10 μM for 48 h</td>
<td>Mouse retinal endothelial cells</td>
<td>Increased proliferation and survival of EC</td>
<td>Transactivation of VEGF receptor Flk1 and PDGF-β receptor by morphine; subsequent MAPK/ERK and Akt phosphorylation</td>
<td>[63]</td>
</tr>
<tr>
<td>Morphine</td>
<td><em>In vitro</em></td>
<td>0.714 mg kg⁻¹ day⁻¹ for 7 days then 1 mg kg⁻¹ day⁻¹ for 7 more days</td>
<td>SCK mammary carcinoma implanted SC into right hind thigh</td>
<td>Increased tumor angiogenesis and tumor growth and metastasis</td>
<td>Morphine upregulates COX-2 and PGE2 levels</td>
<td>[70]</td>
</tr>
<tr>
<td>Morphine</td>
<td><em>In vitro</em></td>
<td>20 mg/kg IP for 2 weeks</td>
<td>Excisional wound injury and Matrigel assays in mice</td>
<td>Impaired angiogenesis</td>
<td>Increased superoxide production by endothelial cells; reduced endothelial progenitor cell mobilization</td>
<td>[78]</td>
</tr>
<tr>
<td>Endomorphin-1; Endomorphin-2; Deltorphin I</td>
<td><em>In vitro</em></td>
<td>10⁻⁸–10⁻⁴ M for 24 h</td>
<td>Human umbilical vein endothelial cells</td>
<td>Reduced capillary tube formation</td>
<td>Reversed by naloxone</td>
<td>[107]</td>
</tr>
<tr>
<td>Morphine</td>
<td><em>In vitro</em></td>
<td>Incubation with 1–10⁶ nM</td>
<td>Human umbilical</td>
<td>Increased apoptosis of</td>
<td>Stimulation of the release of pro-angiogenic factors such as NO and IL-8; transactivation of growth factor receptors; Protecting endothelial cells from oxidative stress</td>
<td></td>
</tr>
</tbody>
</table>
Analgesia (bupivacaine + fentanyl) has been documented to attenuate the postoperative suppression of lymphocyte proliferation and to reduce the pro-inflammatory cytokine response when compared to opioid analgesia after abdominal surgery [84].

There is growing interest in the hypothesis that using regional anesthesia and analgesia (RAA) in cancer surgery patients may reduce the rate of recurrence or metastasis. This is relevant to our topic because one of the mechanisms by which RAA may be protective is that it prevents or reduces the need for intra- and postoperative morphine use. Other proposed mechanisms include preserved postoperative immunity by attenuation of the metabolic, neuroendocrine, and cytokine stress response to surgery and reduced use of general anesthetics.

This hypothesis, supported by animal studies [85, 86], has led to retrospective studies recently carried out to compare recurrence-free survival in patients subjected to general anesthesia and opioid analgesia and patients undergoing surgery with regional anesthesia/analgesia. They showed that regional anesthesia/analgesia significantly reduced the recurrence or metastasis in breast [87] and prostate [88] cancer surgery patients and enhanced survival in colon cancer patients that did not have metastases at the time of surgery [89]. In contrast, epidural analgesia for perioperative pain control was found to offer no advantage for cancer recurrence after colorectal surgery in a separate study [90], and secondary analysis of records from patients randomized to general anesthesia alone or combined general/epidural anesthesia failed to confirm a
difference in prostate cancer biochemical recurrence between epidural and control groups [91]. Clearly, a definitive answer will require prospective, large randomized trials such as [92]; five such studies are currently recruiting patients (clinicaltrials.gov using the search words cancer recurrence regional anesthesia). However, recurrence data and thus interpretation of the results are not expected for several years. In the meantime, prospective studies have attempted to elucidate the mechanism through which regional anesthesia/analgesia might be protective toward cancer recurrence or metastasis in patients. RAA was confirmed to inhibit the surgical stress response, as indicated by lower plasma glucose, cortisol, and C-reactive protein in RAA patients compared to general anesthesia/morphine analgesia—but RAA did not seem to affect the postoperative percent change in pro-angiogenic factors VEGF and prostaglandin E2 [93]. In contrast, regional anesthesia/analgesia decreased circulating TGF-β and IL-1β and attenuated the postoperative increase in lymphangiogenic VEGF-C and in pro-invasion, pro-metastatic, and pro-angiogenic MMP-9 [94, 95]. Lastly, in an ex vivo experiment, the serum from general anesthesia/morphine analgesia patients was shown to promote cancer cell proliferation without affecting cancer cell migration when compared to the serum of patients undergoing RAA [96].

Furthermore, while the data from the prospective studies will provide clinically important results, they will not specifically outline the part that morphine plays in the different anesthesia and analgesia regimens tested. Such data are currently unavailable, and attempts to determine whether opioids promote the development of new tumors in patients via retrospective studies have not shown significant difference [97].

4 Conclusions

Opioids and the most widely used—morphine—have proven direct and indirect effects that can affect the course of cancer; therefore, the question of their use in cancer patients at a crucial time of the disease development is legitimate if appropriate pain management alternatives are available. At present, it is impossible to conclude from the existing data whether altered tumor cell proliferation and invasion, inflammation, angiogen-
esis, and immune response (Fig. 1) will result in harm or benefit. This is due to the complexity of cellular pathways and physiological responses elicited by endogenous and exogenous opioids and, to often, discrepant results from in vitro and in vivo studies. Among the factors influencing the effect of morphine on metastasis are the dose, duration, and continuousness of exposure to morphine, the route of administration, tolerance receptor desensitization and withdrawal effects, central versus peripheral actions, and the variety of models employed which differ in their opioid receptor characteristics and morphine metabolism. Research on the effect of morphine on cancer started several years ago, but is still an extremely active field, and the combination of basic and clinical studies currently underway will no doubt provide the insights that patient care requires in providing.

References


