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REVIEW

The role of morphine in regulation of cancer cell growth

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Abstract Morphine is considered the "gold standard" for relieving pain and is currently one of the most effective drugs available clinically for the management of severe pain associated with cancer. In addition to its use in the treatment of pain, morphine appears to be important in the regulation of neoplastic tissue. Although morphine acts directly on the central nervous system to relieve pain, its activities on peripheral tissues are responsible for many of the secondary complications. Therefore, understanding the impact, other than pain control, of morphine on cancer treatment is extremely important. The effect of morphine on tumor growth is still contradictory, as both growth-promoting and growth-inhibiting effects have been observed. Accumulating evidence suggests that morphine can affect proliferation and migration of tumor cells as well as angiogenesis. Various signaling pathways have been suggested to be involved in these extra-analgesic effects of morphine. Suppression of immune system by morphine is an additional complication. This review provides an update on the influence of morphine on the growth and migration potential of tumor cells.

Keywords Apoptosis · Proliferation · Angiogenesis · Migration · Metastasis

Abbreviations

BAD	Bcl-x_L/Bcl-2-associated death promoter protein
CNS	Central nervous system
COX-2	Cyclooxygenase-2
ECM	Extracellular matrix
EGF	Epidermal growth factor
Erk	Extracellular-regulated kinase
IL	Interleukin
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MMPs	Matrix metalloproteinases
MNTX	Methylnaltrexone
NF-κB	Nuclear factor κB
NO	Nitric oxide
NOS	Nitric oxide synthase
PAI-1	Plasminogen activator inhibitor-1
PAI-2	Plasminogen activator inhibitor-2
PGE_2	Prostaglandin E_2
PI3K	Phosphatidylinositol 3-kinase
RhoA	Ras homolog gene family, member A
ROS	Reactive oxygen species
Src	Non-receptor tyrosine kinase
TIMPs	Tissue inhibitors of metalloproteinases
TNF	Tumor necrosis factor
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator membrane-linked receptor
VEGF	Vascular endothelial growth factor

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Introduction

Morphine, the main component of opium, is perhaps the oldest drug known to man. Pure morphine was isolated in 1803 by Sertürner (Schmitz 1985), and its structure was elucidated 120 years later. Full systemic name of morphine is 7,8-didehydro-4,5-epoxy-17-methyl-(5α, 6α)-morphinan-3,6-diol. Morphine was found to be a particularly good analgesic and sedative, far more effective than crude

opium. Morphine was shown to exert its action through opioid receptors (μ, δ, and κ) localized in the brain (Pasternak 1993; Reisine and Bell 1993; Harrison et al. 1998; Kieffer 1999; Kieffer and Gaveriaux-Ruff 2002). However, morphine binds to the μ-opioid receptor with nearly two orders of magnitude greater affinity compared with the other two opioid receptors (Zadina et al. 1994). Although morphine acts directly on the central nervous system (CNS) to relieve pain, its activity on peripheral tissues is responsible for many of the secondary complications. Besides its strong analgesic effect, morphine exerts a number of adverse side-effects, including addiction, tolerance, respiratory depression, immunosuppression, and constipation. The lack of equally strong painkillers is the reason that despite the drawbacks mentioned above, morphine is still the most commonly used analgesic for management of severe pain, including cancer pain (Mantyh 2006). Administration of morphine to cancer patients gave evidence that apart from its analgesic action, morphine can significantly alter tumor growth. In the last decade, numerous studies employing cancer cell lines and experimental animals have been performed to reveal complex mechanisms by which morphine affects tumor cells. Whereas the pharmacology and function of opioids in the CNS have been extensively characterized, still little is known about their effect on cancer cells. The results obtained so far are conflicting. In the past, morphine was reported to increase the proliferation of endothelial and tumor cells (Simon and Arbo 1986; Moon 1988; Ishikawa et al. 1993; Gupta et al. 2002). On the other hand, morphine and other opioids were also found to promote tumor cell death (Maneckjee et al. 1990; Yeager and Colacchio 1991; Page et al. 1993; Hatzoglou et al. 1996; Sueoka et al. 1996, 1998). Several reviews have covered this important research area from different perspectives (Rasmussen et al. 2002; Fichna and Janecka 2004; Tegeder and Geisslinger 2004; Chen et al. 2008). This review will focus on the latest findings on the influence of morphine on tumor cell proliferation, apoptosis, angiogenesis, and migration.

Effect of morphine on tumor growth

Despite extensive research, it is still not well understood whether morphine itself directly modifies the growth of tumor cells. Some authors postulate that morphine can promote tumor growth and reduce the survival rate of tumor-bearing animals due to immunosuppression, since the negative effects of morphine and other opioids on the immune system are well established (Odunayo et al. 2010). On the other hand, multiple research data indicate that morphine can accelerate or inhibit cancer cell growth in vitro and in vivo by different mechanisms.

High concentrations of morphine were shown to reduce the growth of tumors. Tegeder et al. (2003) reported that morphine inhibited tumor cell proliferation at concentrations of >10 μM. In nude mice, morphine significantly reduced the growth of MCF-7 and MDA-MB-231 tumors. Intermittent injections of morphine decreased the growth of tumors in a rat model of metastasizing colon cancer (Yeager and Colacchio 1991).

On the other hand, morphine was shown to trigger stimulation of human glioblastoma T98G cell proliferation (Lazarczyk et al. 2010). Gupta et al. (2002) demonstrated that morphine, in clinically relevant doses, promoted tumor neovascularization in a human breast tumor xenograft model in mice, leading to increased tumor progression.

The discrepancies in results may be due to the differences in administered doses or/and the mode of administration (systemic versus localized). These examples, as well as some other reports (Maneckjee et al. 1990; Harimaya et al. 2002; Sasamura et al. 2002), showed that tumor suppression occurs after chronic high doses of morphine, while tumor-enhancing effects with morphine occur after a single dose or low daily doses (Zong and Pollack 2000). This dual concentration-dependent effect, i.e., mitogenesis at low and growth inhibition at higher concentrations was observed both in vitro and in vivo.

The lack of effects of morphine on the proliferation of colon cancer HT-29 cells (Zagon et al. 1996; Nylund et al. 2008) and on breast cancer MCF-7 cells was also reported (Janecka et al. 2004).

Recently, it was demonstrated that the μ-opioid receptor regulates cancer progression in animal models (Moss and Rosow 2008; Wang et al. 2009; Singleton and Moss 2010). The μ-opioid receptor-knockout mice were shown not to develop significant tumors when injected with Lewis lung cancer cells as did the wild-type controls (Mathew et al. 2009). Silencing the expression of the μ-opioid receptor in Lewis lung cancer cells inhibited lung metastasis in wild-type mice by about 75%. Finally, infusion of the μ-opioid receptor antagonist, methylnaltrexone, markedly attenuated tumor growth in wild-type mice treated with Lewis lung cancer cells by up to 90% (Mathew et al. 2009). These experimental data strongly support the hypothesis that the μ-opioid receptor promotes tumor growth and metastasis.

Possible mechanisms of morphine action mediated through the opioid receptors are demonstrated in Fig. 1 and through the non-opioid pathways in Fig. 2. The summary of in vivo growth-promoting and growth-inhibiting effects of morphine is presented in Table 1.

Effect of morphine on tumor apoptosis

Apoptosis is an active process of controlled cell death in the development and maintenance of tissue homeostasis.

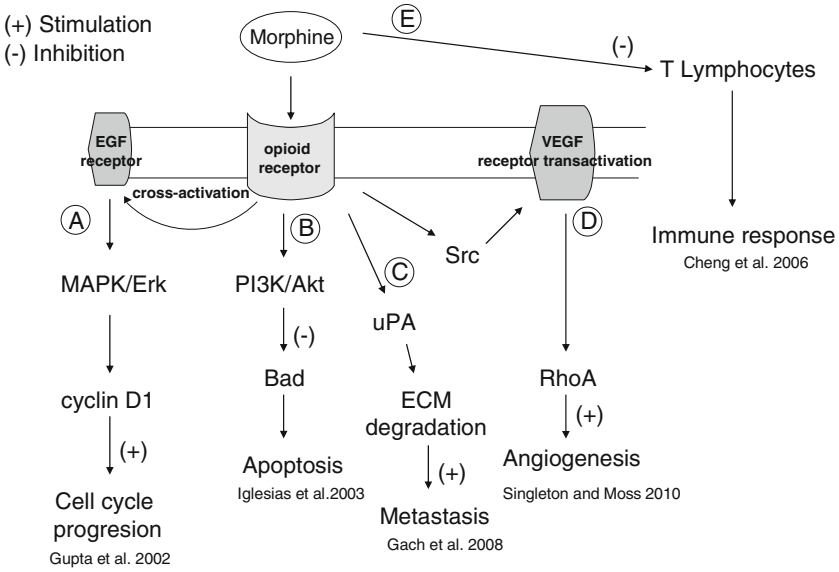

Fig. 1 Possible mechanisms of opioid receptor mediated influence of morphine on tumor growth. Morphine binds to the μ-opioid receptor and **a** stimulates MAPK/Erk pathway, which results in the cell cycle progression; **b** activates PI3K/Akt pathway mediating anti-apoptotic effects; **c** up-regulates uPA expression and secretion promoting metastasis; **d** transactivates VEGF receptors and induces angiogenesis; **e** suppresses the function of T lymphocytes, leading to immunosuppression

Apoptosis is controlled by two main pathways, the intrinsic or the mitochondrial-mediated pathway (Green and Reed 1998) and the extrinsic or death receptor-mediated pathway (Ashkenazi and Dixit 1999). Mitochondrial-mediated apoptosis is controlled by Bcl-2 family of proteins (Cory and Adams 2002; Cory et al. 2003). The death receptor-mediated pathway is initiated by the ligation of cell death ligands with their death receptors (Sartorius et al. 2001). Caspase-3 is activated in both apoptotic pathways and plays central role in the execution phase of cell apoptosis (Ashkenazi and Dixit 1999; Hengartner 2000; Fulda and Debatin 2006).

Apoptosis is usually deregulated in cancer cells, and this deregulation can contribute to uncontrollable proliferation and tumor growth (Hanahan and Weinberg 2000; Hengartner 2000; Kaufmann and Hengartner 2001). Morphine was shown to induce the apoptosis of human endothelial cells (Hsiao et al. 2009) and of T lymphocytes and macrophages (Kapasi et al. 2004). Morphine in high concentrations (10^{-3}–10^{-6} M) was demonstrated to induce apoptotic cell death in human tumor cell lines (Kawase et al. 2002; Hatsukari et al. 2003). In other studies, the pro-apoptotic effect of morphine in human tumor cell lines was observed at clinical concentration (10^{-8} M) (Smith et al. 1999; Hatsukari et al.

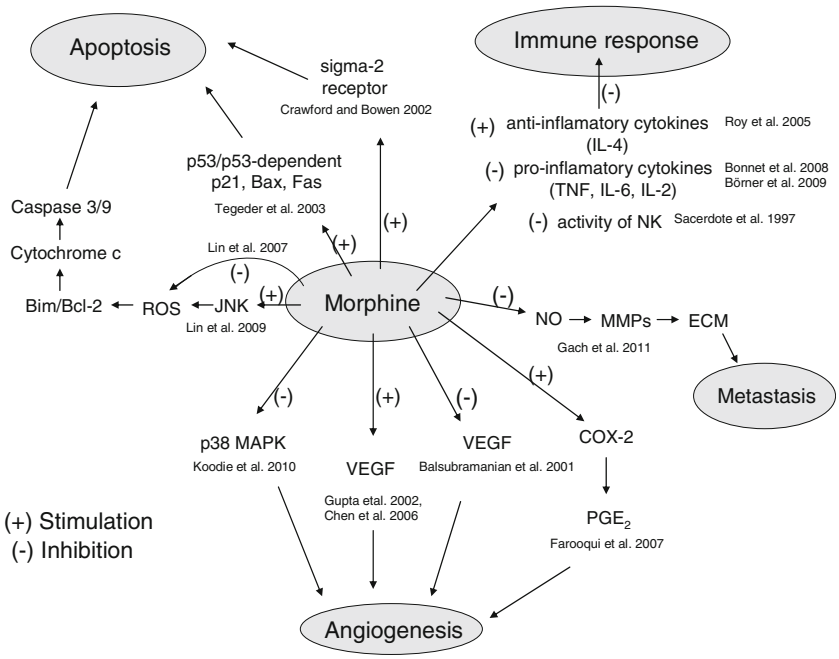

Fig. 2 Possible pathways, other than through the opioid receptors, by which morphine influences cancer progression and suppression

Table 1 Summary of in vivo growth-promoting and growth-inhibiting effects of morphine

Cell line/tumor type	In vivo effects	In vivo dose/time	Suggested mechanism	Antagonist/inhibition	Ref.
Ehrlich carcinoma injected s.c. in the left thigh of BALB/c mice	Stimulation of angiogenesis	0.714 mg/kg/day for 7 days (equivalent to 50 mg per day for a 70 kg human)			Ustun et al. 2010
Colon cancer cells injected i. p. in Fisher 344 rats	Inhibition of tumor growth and metastasis (significant decrease in the hepatic tumor burden)	20 mg/kg/day s.c., the day before and for 2 days after colon cancer cell inoculation	Enhancement of NK cell activity at the time of tumor cell injection		Yeager and Colacchio 1991
MCF-7, MDA-MB231 breast cancer or HT-29 colon cancer cells injected s. c. in NMRI-nu/nu mice	Inhibition of tumor growth (MCF-7 and MDA-MB231) no effect on HT-29 tumors	10–30 mg/kg/day i.p. for 3 weeks (stepwise 10, 20, and 30 mg/kg/day i.p. for the first, second, and third week)	Inhibition of tumor growth through a p53 dependent mechanism (up-regulation of p53-dependent p21, Bax, Fas)	Naloxone increased the growth-inhibitory effects of morphine	Tegeder et al. 2003
EL-4 leukemia in C57Bl6 mice, sarcoma 180 in ddY mice	Increase of tumor growth	10 mg/kg/day s.c. for 10 days	General immunosuppressive effect	Naloxone itself had no significant effect. The effect was inhibited by preadministration of naloxone	Ishikawa et al. 1993
Walker 256 carcinosarcoma cells injected i. v. in Sprague–Dawley rat	Increase of the number metastases	5 mg/kg single dose	Suppresion of NK cells	Naloxone itself had no significant influence on the number of metastases. The effect was inhibited by preadministration of naloxone	Simon and Arbo 1986
B16-BL6 melanoma cells injected into hindpaw in C57Bl mice	Inhibition of tumor growth and metastasis (decreased number of tumor nodules in the lung)	5 and 10 mg/kg/day s.c. for 6 days, starting 16 days after cancer cells injection	Pain reduction and blockade of pain signals		Sasamura et al. 2002
Colon 26-L5 carcinoma cells injected i.v. in BALB/c mice	Inhibition of tumor metastasis (reduction of the number of tumor colonies in the lung)	10 mg/kg/day i.p. for 6 days, starting on day 2 after i.v. inoculation of tumor cells	Inhibition of adhesion and invasion of cancer cells (inhibition of MMP-2 and 9 production)		Harimaya et al. 2002
MCF-7 cells injected into the mammary fat pad of nude mice	Increase of tumor growth associated with increased angiogenesis (neovascularization, increased microvessel density, higher vessel number, increased total length, and more vessel branching)	0.714 mg/kg mouse/day for first 15 days and then 1.43 mg/kg mouse/day (equivalent to 50 mg and 100 mg morphine per day, respectively, for a 70 kg human)	Activation of PI3K/Akt and Erk pathways, resulting in inhibition of apoptosis and promotion of cell cycle progression	Naloxone itself had no significant effect on angiogenesis. The anti-angiogenic effect of morphine was not inhibited by naloxone	Gupta et al. 2002
MADB-100 mammary adenocarcinoma cells injected i.v. in Fisher 344 rats, 5 h after surgery	Inhibition of surgery-induced increase in metastasis	5 mg/kg 3 doses (pre, post and 5 h post surgery)	Inhibition of postoperative pain and stress-induced immunosuppression		Page et al. 1993
Murine CCL-11 sarcoma cells injected into femur of the right leg in C3H/HeJ mice	Morphine did not alter tumor burden	50, 25, 7.5, 2.5 mg/ml implanted (s.c.) for 7 days following injection of CCL-11 cells into the femur			King et al. 2007

Table 1 (continued)

Cell line/tumor type	In vivo effects	In vivo dose/time	Suggested mechanism	Antagonist/inhibition	Ref.
Lewis lung carcinoma cells injected s.c. in nude mice	Reduction of tumor cell-induced angiogenesis and tumor growth	A continuous slow-release morphine pellet resulting in morphine plasma levels within 250–400 ng/ml	Suppression of the hypoxia-induced mitochondrial p38 mitogen-activated protein kinase (MAPK) pathway	The effect was abolished with naltrexone	Koodie et al. 2010
TC-1 cells injected s.c. into the right leg of C57BL/6J mice	Dose-dependent increase of tumor growth	10 mg/kg i.p., twice daily from day 10 before tumor injection	Immunosuppression (μ-opioid receptor mediated suppression of T lymphocytes proliferation, promotion of apoptosis of T lymphocytes through Bcl-2 and Bax apoptosis-related molecules)		Cheng et al. 2006

2007). However, significant differences between cell lines were found. For example, morphine produced a higher number of necrotic cells in the MCF-7 breast cancer cell line than in the A549 lung cancer cell line (Hatsukari et al. 2007).

Consistent with some previous studies (Tegeder et al. 2003), it was recently demonstrated that chronic high-dose morphine treatment was able to cause apoptotic cell death of SH-SY5Y cells in an opioid receptor-independent manner (Lin et al. 2009). A pivotal role in this process was played by c-Jun N-terminal kinase (JNK). Activation of JNK by morphine led to reactive oxygen species (ROS) generation and induced cytochrome c release and caspase-9/3 activation through enhancement of expression of the pro-apoptotic protein Bim and reduction of expression of the anti-apoptotic protein Bcl-2. All of these effects of morphine could be suppressed by the JNK inhibitor. The key role of the JNK pathway in morphine-induced mitochondria-dependent apoptosis was further confirmed by the observation that decreased levels of JNK in cells transfected with specific small interfering RNA resulted in resistance to the pro-apoptotic effect of morphine.

It can be concluded that morphine can induce apoptosis and inhibit the growth of cancer cells by activating different signal pathways. Apart from the mitochondrial pathway mentioned above, apoptosis of MCF-7 cells was shown to be mediated by a novel sigma-2 receptor and p53 and caspase-independent pathway (Crawford et al. 2002; Crawford and Bowen 2002). CNE2 human epithelial tumor cell line was reported to undergo apoptosis by the activation of the κ-opioid receptor via the phospholipase pathway (Diao et al. 2000).

The growth-inhibitory or apoptosis-inducing effects of morphine might be directly associated with morphine tolerance (Wu et al. 1999; Mao et al. 2002) or receptor desensitization, as assessed by a lack of morphine-stimulated GTP-ase activity at concentrations that inhibit tumor growth (Tegeder et al. 2003). Drugs that prevented the development of morphine tolerance in rats also prevented cell death (Mao et al. 2002). This close association between apoptosis and receptor desensitization suggests that receptor internalization may be the key event in initiating opioid-evoked cell death.

On the other hand, evidence of the anti-apoptotic activity of morphine is also accumulating (Suzuki et al. 2003). It was shown that morphine can antagonize the pro-apoptotic activity of a well-known anti-tumor drug, doxorubicin, in neuroblastoma SH-SY5Y cells (Lin et al. 2007). The effect could not be reversed by naloxone, indicating a non-opioid receptor-mediated signaling pathway. Further studies showed that morphine attenuated doxorubicin-induced apoptosis by the inhibition of ROS generation and mitochondrial cytochrom c release, as well as by blockade of nuclear factor κB (NF-κB) transcriptional activation. NF-κB is a ubiquitous nuclear transcription factor that plays a major regulatory role in apoptosis and inflammation. Iglesias et al. (2003) demonstrated that in SH-SY5Y neuroblastoma cells, morphine (10^{-7}–10^{-5} M) promoted cell survival after serum deprivation without inducing cell proliferation, and this effect was fully reversed by naloxone. It was shown that in neuronal cells, μ-opioid agonists do not directly induce apoptosis, but are able to activate the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signal transduction pathway, thus leading to cell survival.

Effect of morphine on angiogenesis

Successful tumor growth depends on many aspects, of which the most important are proliferation of tumor cells and angiogenesis or the formation of new blood vessels which

are necessary for metastasis (Carmeliet and Jain 2000). To date, very few studies have investigated the effect of morphine on tumor cell-induced angiogenesis. Gupta et al. (2002) showed that at clinically relevant concentrations, morphine stimulated human microvascular endothelial cell proliferation and angiogenesis in vitro, and that in vivo these effects translated into enhanced tumor neovascularization in the MCF-7 breast cancer model. In addition, this group demonstrated that morphine promoted activation of vascular endothelial growth factor (VEGF) receptor and increased metastasis and reduced survival in animal model of hormone-dependent breast cancer (Chen et al. 2006; Farooqui et al. 2007). However, the opioid receptor antagonist, naloxone, did not inhibit the pro-angiogenic activity of morphine, indicating that the effect was not mediated by the typical opioid receptors.

On the other hand, Singleton and Moss (2010) demonstrated that μ-opioids (morphine) transactivate the VEGF receptors and promote angiogenesis. The μ-opioid receptor antagonist methylnaltrexone blocks opioid-induced angiogenesis in cultured human endothelial cells.

It was postulated that the pro-angiogenic activity of morphine is connected with the stimulation of mitogen-activated protein kinase (MAPK) signaling pathway via G protein-coupled receptors and nitric oxide (NO). Chronic morphine treatment increased the levels of nitric oxide synthase (NOS), NO, and cyclooxygenase-2 (COX-2) in mouse kidney (Stefano et al. 1995; Arerangaiah et al. 2007). NO stimulated the enzymatic activity of COX-2 (Salvemini et al. 1993), and activated COX-2 in turn increased prostaglandin E_2 production (Salvemini et al. 1993, 1994; Nedelec et al. 2001; Birnbaum et al. 2005). Prostaglandin E_2 promoted angiogenesis and tumor progression (Griffin et al. 2002; Leahy et al. 2002; Chang et al. 2004). The role of COX-2 in cancer progression was further studied by Farooqui et al. (2007). Two weeks of chronic morphine treatment of experimental mice with highly invasive SCK breast cancer model stimulated COX-2, prostaglandin E_2, and angiogenesis, which was accompanied by increased tumor weight, increased metastasis, and reduced survival. It was postulated that products of COX-2, such as prostaglandin E_2 produced in tumor cells, act on the tumor endothelium and promote angiogenesis. Notably, morphine induced a much stronger expression of COX-2 in tumor cells than in endothelial cells. Co-administration of COX-2 inhibitor, celecoxib, prevented morphine-induced promotion of angiogenesis and tumor growth. Therefore, morphine-induced tumor growth may in part be due to the up-regulation of COX-2 and prostaglandin E_2-mediated stimulation of angiogenesis. It was also shown that morphine did not have any effect on MCF-7 human breast cancer cell proliferation in vitro (Farooqui et al. 2006) but stimulated MCF-7 breast tumor growth by promoting angiogenesis (Gupta et al. 2002). That means that morphine by itself did not modulate tumor cell growth in culture, but due to up-regulation of COX-2 in tumor cells, stimulated angiogenesis in vivo, resulting in increased tumor growth and metastasis. The pro-angiogenic activity of morphine was also reported by Ustun et al. (2010).

Contradictory data on the effect of morphine on angiogenesis were also published. It is well known that when the solid tumor grows, new tumor cells are localized further away from their vascular supply, and low oxygen tensions or hypoxia stimulate tumor cells to secrete pro-angiogenic factors (Folkman and D'Amore 1996). A potent pro-angiogenic factor secreted by hypoxic tumor cells within developing solid tumor is vascular endothelial growth factor (VEGF). This factor promotes new blood vessel formation to sustain tumor growth and initiates endothelial cell proliferation and migration (Brekken and Thorpe 2001; Ferrara 2004). Balasubramanian et al. (2001) reported that morphine inhibited hypoxia-induced VEGF secretion in rat cardiomyocytes and human umbilical vein endothelial cells and therefore attenuated the ability of hypoxic tumor cells to induce angiogenesis. Most recently, the same effect was observed in a murine Lewis lung carcinoma tumor model (Koodie et al. 2010). Morphine was administered to mice at the clinically relevant analgesic doses by implantation of continuous slow-release pallets. Morphine plasma level within 250–400 ng/mL was sufficient to significantly reduce tumor cell-induced angiogenesis and tumor growth. The effect of morphine was abolished by concomitant administration of opioid antagonist, naltrexone, and also in the μ-opioid receptor knockout mice, supporting the involvement of classical opioid receptors in the process. The authors also demonstrated that the inhibitory effect of morphine was mediated through the suppression of the hypoxia-induced mitochondrial p38 MAPK pathway.

Much earlier, it was shown that high concentrations of morphine (10 mg/mL of plasma) inhibited angiogenesis in the chick chorioallantoic membrane assay (Pasi et al. 1991). However, morphine is cytotoxic to endothelial cells at such high concentrations (Gupta et al. 2002), so the observed effect could have been non-specific. In wound healing experiments in mice, systemic administration of high-dose morphine resulted in impared mobilization of endothelial progenitor cells and angiogenesis, delaying the healing process. Consistent with the in vivo experiment, in cultured endothelial cells, morphine reduced capillary tube formation in a concentration-dependent manner (Lam et al. 2008).

Effect of morphine on migration and invasiveness of tumor cells

A major feature of cancer cells is their ability to migrate and settle in surrounding or distant tissues. Most cancer

deaths and the main cause of failure in cancer treatment is not due to growth of the primary tumor but results from its intensive spread (metastasis) to secondary sites (Engbring and Kleinman 2003; Widel and Widel 2006). The critical step of cancer dissemination is migration of cancer cells through the extracellular matrix (ECM). Indispensable in this process is activation of urokinase plasminogen activator system, which includes a serine proteinase, urokinase plasminogen activator (uPA), two inhibitors, PAI-1 and PAI-2, and the membrane-linked receptor (uPAR) (Duffy and Duggan 2004), plasmin and matrix metalloproteinases (MMPs) (Mignatti and Rifkin 2000) capable of degrading the ECM.

It was shown that uPA, PAI-1, and uPAR levels are up-regulated in most types of cancers (Shapiro et al. 1996). The effect of morphine on uPA levels was studied in vitro by several authors. Gach et al. (2009) showed that morphine caused a markedly increased secretion of uPA in MCF-7 breast cancer cells, which correlated well with up-regulation of uPA and uPAR mRNA levels. Naloxone reversed morphine-induced up-regulation of uPA and uPA mRNA levels confirming the involvement of the opioid receptors in this process. A similar result was reported by Nylund et al. (2008) who demonstrated that morphine stimulated uPA secretion in HT-29 colon cancer cells.

The MMPs constitute a family of zinc-dependent endopeptidases, whose primary function is remodeling of components in the ECM (Engbring and Kleinman 2003; Widel and Widel 2006; Jespersen et al. 2009). Among the different MMPs involved in degradation of ECM in cancer cells, it seems that MMP-2 (gelatinase A) and MMP-9 (gelatinase B) play a crucial role in cancer invasion because of their ability to degrade type IV collagen, a major component of basement membranes (Widel and Widel 2006). The activity of MMPs is strictly regulated at the transcription and translation levels by endogenous inhibitors, including α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs). Several studies have shown that MMP expression and activity were increased in several tumor types, especially breast and lung cancer. The up-regulated levels of MMPs correlated well with tumor stage, increased invasion, and potential metastasis (Lynch and Matrisian 2002; Widel and Widel 2006). Morphine was found to significantly reduce experimental lung metastasis and invasion of colon 26-L5 cells by inhibiting adhesion and migration of these cells to ECM (Harimaya et al. 2002). In the mouse fibrosarcoma cell line, WEHI 164, activity of MMP-2 was shown to be under control of NO system and was attenuated by low doses of morphine in a non-opioid receptor-related manner (Shariftabrizi et al. 2006). In the MCF-7 cell line, morphine inhibited expression and secretion of MMP-2 and -9 in time- and concentration-dependent manner, and the process was not mediated by opioid receptors but was also under the control of the NO system (Gach et al. 2011).

Effect of morphine on immune system

Disorders in the immune system can result in disease, including inflammatory diseases and cancer. Morphine mediates its analgesic effect by the μ-opioid receptor. The expression of the μ-opioid receptor gene in neuronal cells is regulated by cytokines, released from the cells of the immune system. On the other hand, μ-opioid receptor is also expressed in the cells of immune system, such as lymphocytes and macrophages (Börner et al. 2007). This points at the importance of interactions between opioid and immune system (Kraus 2009). The fact that morphine can inhibit numerous immune cell functions and cause immunosuppression is well known (Peterson et al. 1993; Sacerdote et al. 1997; Eisenstein and Hilburger 1998). In particular, morphine decreases the activity of natural killer cells which play a major role in the rejection of tumors (Sacerdote et al. 1997) and increases the susceptibility of animals and humans to bacterial and viral infections (Yeager et al. 1995; Risdahl et al. 1998; MacFarlane et al. 2000). It was also shown that morphine inhibits production of pro-inflammatory cytokines (e.g., TNF, IL-6) in monocytes (Bonnet et al. 2008), modulates the T helper cell balance by inducing up-regulation of an anti-inflammatory IL-4 mRNA (Roy et al. 2005) and produces immunosuppression by inhibition of transcription of IL-2 in activated lymphocytes T (Börner et al. 2009). However, future research is necessary to provide clearer understanding of the cellular and molecular targets of morphine action within the immune system.

Conclusions

For more than two decades, researchers have been trying to document the effects that morphine exerts on tumor cells. It has been recognized that morphine can affect tumor growth by either directly acting on the tumor cells or by directly acting on the endothelial cells or on the CNS-mediated secretion of growth factors that may alter tumor microenvironment. Suppression of immune system by morphine can be an additional complication. However, the results obtained both in in vitro and in vivo studies are conflicting. On one hand, morphine was shown to induce tumor growth, inhibit apoptosis, promote angiogenesis and migration of tumor cells, but on the other hand, pro-apoptotic and anti-angiogenic properties of morphine were also demonstrated. Performed studies were very heterogenic due to the different doses of morphine applied, and the effects may vary when the experiments were performed in vivo or on isolated cells. The dose and route of administration of morphine might be critical factors that need to be taken into consideration in clinical settings. Contradictory results that

have been obtained induce further studies. The final answer to the question whether morphine is an inhibitor of tumor growth or whether it promotes cancer is still to be sought. The effects of morphine can also depend on a cancer type, since different cancer cells can overexpress certain enzymes which are possible morphine targets.

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