

## Chapter 2

# Interaction of Naloxone and Estrogen Receptor in Breast Cancer

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**Abstract** Majority of breast cancers are estrogen receptor (ER) positive. Due to resistance to known ER-based therapies, novel treatment targets and drugs are required to effectively treat ER-positive breast cancer. Opioids are often used to treat pain in breast cancer and promote tumor growth and metastases in rodent studies. Opioid receptor (OR) antagonists, such as naloxone, naltrexone and methylnaltrexone inhibit cancer progression and metastases. All three antagonists share structural similarities with the estrogen, 17 $\beta$ -estradiol (E2), and are therefore capable of binding to ER. Naloxone inhibits E2-induced human MCF-7 breast cancer cell proliferation and MAPK/ERK signaling. Additionally, naloxone also attenuates the activation of membrane bound/cytoplasmic ER and phosphorylation of the epidermal growth factor receptor. Naloxone blocks the E2-induced ER activation by precluding its binding to the co-activator and by directly competing with E2 for binding to ER. In addition to these direct interactions with ER, naloxone prevents the cross-talk of ER with mu opioid receptor (MOR), suggesting that activation of MOR may contribute to E2-induced ER activation. Since naloxone and structurally similar OR antagonists inhibit cancer progression and metastases, OR antagonists can be potentially developed for breast cancer treatment.

**Keywords** Angiogenesis • Breast cancer • EGF receptor • Estrogen receptor • G protein coupled receptors • Methylnaltrexone • Naloxone • Naltrexone • Opioid receptor • Therapy

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## Abbreviations

E2	17 $\beta$ -estradiol
AF1	activation-function 1
AF2	activation-function 2
AI	aromatase inhibitor
cAMP	cyclic adenosine monophosphate
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ERE	estrogen response element
Gi-GPCRs	inhibitory regulated-G protein coupled receptors
LBD	ligand-binding domain
MNTX	methylnaltrexone
MAPK/ERK	mitogen activated protein kinase/extracellular signal-regulated kinase
Nal	naloxone
NTX	naltrexone
NOP	nociceptin/orphanin FQ receptor
OR	opioid receptor
PI3K	phosphatidylinositol 3-kinase
Akt	protein kinase B
SERMs	selective ER modulators
VEGFR2	vascular endothelial growth factor receptor 2
DOR	$\delta$ opioid receptor
KOR	$\kappa$ opioid receptor
MOR	$\mu$ opioid receptor

## 2.1 Interaction of Naloxone and Estrogen Receptor in Breast Cancer

In the developed world breast cancer is the most common malignancy amongst women. It is estimated that in the United States (US) alone, about 12% of women, which amounts to one out of eight women, will develop invasive breast cancer in their life-time ([www.breastcancer.org](http://www.breastcancer.org)). An alarming number of new cases of invasive and non-invasive breast cancer (230,480 and 57,650, respectively) were to be diagnosed in women and 39,520 women were expected to die of breast cancer in 2011 in the US. In spite of a slow decline in the breast cancer incidence, (about 2%) due to increased awareness, early detection and treatment, breast cancer remains the second most common malignancy in women in the US. About 80% of breast cancers are estrogen-receptor positive. Therefore, strategies to attenuate estrogen receptor (ER) activity are critical to cure breast cancer.

## 2.2 Role of Estrogen Receptor

The ER is a member of the nuclear steroid-hormone receptor superfamily (Mangelsdorf et al. 1995; Hall et al. 2001). The two isoforms of ER, ER $\alpha$  and ER $\beta$ , have a high degree of homology, particularly in their ligand and DNA binding domains and exhibit the characteristic features common to intracellular nuclear receptors (Hall et al. 2001). ER $\alpha$ - and ER $\beta$ -knockout mice display different phenotypes. Female ER $\alpha$ -knockout mice show complete estrogen insensitivity in the reproductive organs, and have stunted mammary glands in addition to other phenotypic changes, whereas, female ER $\beta$ -knockout mice have limited ovarian function (Lubahn et al. 1993; Krege et al. 1998; Dupont et al. 2000; Korach et al. 2003). Both isoforms play a critical role in the normal and malignant biology of the breast, where, ER $\alpha$  is the predominant form in neoplastic breast epithelium, while ER $\beta$  is more common in normal breast tissue (Khan et al. 1994; Hall et al. 2001). It is suggested that ER $\beta$  may regulate ER $\alpha$  activation, by decreasing cellular sensitivity to estrogens (Hall and McDonnell 1999).

ER $\alpha$ -dependent breast cancer progression has been a subject of intense investigation to develop targeted therapies. About one third of breast cancers are ER $\alpha$ -negative and difficult to treat, but about 65% are ER-positive (Howe and Brown 2011). Several ER $\alpha$ -based drugs are suggested to have a preventive effect on breast cancer in women at moderate- to high-risk of developing breast cancer based on a large phase III clinical trial (Cuzick et al. 2011; Vogel et al. 2010). Drugs tested included the aromatase inhibitor (AI), exemestane and selective ER modulators (SERMs), tamoxifen, raloxifene and lasofoxifene. These drugs reduce the risk of developing breast cancer and are also used to treat ER $\alpha$ -positive breast cancer. In spite of the promising effect of these therapies, patients with breast cancer develop resistance to therapy. Epidermal growth factor receptor family, ErbB family, which includes the epidermal growth factor receptor (EGFR), has been suggested to play an important role in development of resistance to hormonal therapy (Massarweh et al. 2008; Arpino et al. 2004; Emde et al. 2011). Thus a sub-set of ER-positive breast cancer's therapeutic outcomes are challenged by activation of alternative growth factor signaling pathways.

## 2.3 Mechanism of Action of ER

ERs display the characteristic features of nuclear hormone receptors. Structurally, ER $\alpha$  acts in a ligand-dependent as well as ligand-independent way (Gronemeyer et al. 2004; Nilsson et al. 2011). The amino terminal activation-function 1 (AF1) activates ligand-independent transcription and the carboxy terminal, activation-function 2 (AF2) region consists of a multifunctional ligand-binding domain (LBD). The central region consists of the DNA-binding domain, which binds to the specific

sequence of DNA on the estrogen response element (ERE) for transcriptional activation. The transcriptional activity of ER further depends upon activating and repressing co-regulators in the nucleus.

ER $\alpha$  exists in an inactive form in the cytoplasm and nucleus of the cell. Ligand binding induces a conformational change resulting in homodimerization and nuclear translocation, followed by binding to the ERE and transcriptional activation (Nilsson et al. 2011). In addition to the classical activation by specific ligands, cytoplasmic ERs are phosphorylated by activated growth factor receptors, such as EGFR. In turn, activated cytoplasmic ERs stimulate mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt) phosphorylation directly as well as by a cross talk with inhibitory regulated-G protein coupled receptors (Gi-GPCRs) (Hammes and Levin 2007; Wu et al. 2011). However, cytoplasmic activation of the ERs does not promote breast cancer growth, but stimulates endothelial cell-specific activities. In a tumor microenvironment, activated growth factor receptors and GPCRs may therefore further promote ER-induced cell survival and proliferation in breast cancer, by contributing to increased angiogenesis and perhaps increased resistance to hormonal therapy.

## 2.4 Opioid Receptors

Gi-GPCR family includes opioid receptors (OR). The significance of OR activity is two-fold in cancer biology because: (1) OR agonists such as morphine are often used to treat severe pain in cancer and (2) opioid-induced cell survival and proliferation may contribute to cancer progression and metastases directly and by promoting angiogenesis (Gupta et al. 2002, 2007; Stephenson and Gupta 2006; Farooqui et al. 2007; Singleton et al. 2006).

There are four different classes of classical ORs  $\mu$ ,  $\delta$ ,  $\kappa$  (MOR, DOR, and KOR, respectively) and nociceptin/orphanin FQ receptor (NOP) (Finley et al. 2008; Gupta et al. 2007; Stephenson and Gupta 2006). These receptors are coupled to Gi/Go type of G-protein and inhibit adenylyl cyclase activity resulting in a decrease in the basal production of cyclic adenosine monophosphate (cAMP). However, chronic activation of ORs may lead to superactivation of adenylyl cyclase and increased cAMP (Gupta et al. 2007). Depending upon its binding affinity each opioid is a selective agonist for a specific receptor, whereas, Naloxone (Nal) is a non-selective antagonist.

## 2.5 Possible Role of Naloxone in Cancer

Opioid receptor antagonists, such as Nal and Naltrexone (NTX), were shown to inhibit the growth of neuroblastoma and mammary tumors in vivo, almost three decades ago (Aylsworth et al. 1979; Zagon and McLaughlin 1983b; Tsunashima 1982).

These observations support the antitumor activity of OR antagonists, but raise the possibility that these antagonists may attenuate the analgesic ability of endogenous opioids and exogenously administered opioid analgesic drugs. However, more recent studies suggest an anti-nociceptive effect of a low dose of naloxone by itself and/or co-administered with opioids (Lunzer et al. 2007; Power 2011).

The MCF-7 cell line is a widely studied human breast cancer tumor model that is estrogen dependent. Several studies with MCF-7 cells suggest the modulatory effects of opioids and their receptor(s) on estrogen and its receptor(s) and vice versa (Table 2.1) (Cadet et al. 2002; Panagiotou et al. 1998; Sinchak and Micevych 2001). In earlier studies, however, opioid-induced cell proliferation was Nal-insensitive in vitro, yet Nal potently inhibited tumor growth in vivo (Gupta et al. 2002; Tegeder et al. 2003; Maneckjee and Minna 1992; Kugawa et al. 1998; Hatzoglou et al. 1996a; b). We found that morphine stimulated angiogenesis and human MCF-7 breast cancer cell tumor xenografts in nude mice, whereas, Nal inhibited tumor growth in this model (Gupta et al. 2002). Intriguingly, more recent studies from our laboratory showed that 17  $\beta$ -estradiol (E2)-induced MCF-7 breast cancer cell proliferation was inhibited by 100 nM Nal, but not by morphine (Farooqui et al. 2006). Together, these observations suggest an interaction of Nal with the E2-stimulated pathways and/or antagonism of constitutively activated ORs in MCF-7 cells. Thus, morphine appears to promote cancer growth by promoting angiogenesis, and Nal attenuates breast cancer progression by acting directly on the cancer cells.

## 2.6 Structural Similarity Between ER Agonists/ER Antagonists and OR Antagonists

We observed that the phenolic hydroxyl group required for the binding of ER ligands to ER is also present in Nal (Farooqui et al. 2006; Fig. 2.1). The phenolic hydroxyl group is a common feature of several OR antagonists including NTX and methylnaltrexone (MNTX). Superimposition of energy-minimized conformations of E2 (magenta), Nal (cyan), and MNTX (yellow), show the overlap of the phenolic hydroxy-bearing aromatic ring (solid white arrow) of all the compounds (Fig. 2.1a). The N-allyl and cyclopropylmethyl substituents of Nal and MNTX respectively, occupy the same region in space as the D-ring of the steroidal E2 (Fig. 2.1a). Superimposition of energy-minimized conformations of E2 (green), NTX (yellow), MNTX (magenta) and 4-hydroxytamoxifen (cyan), depict the overlap of the phenolic hydroxy-bearing aromatic ring (solid red arrow) (Fig. 2.1b). The N-substitution of NTX and MNTX occupies the same region of space as the D-ring of E2 (green arrow), and may be responsible for their action as antagonists of ER. This conclusion is corroborated by the observations that Nal inhibits the binding of E2 to ER $\alpha$  in vitro (Farooqui et al. 2006).

**Table 2.1** Effects of opioid receptor antagonists on cancer model systems

Opioid receptor antagonist	Dose/route of administration	Model system	Outcome	Reference
<i>Naloxone</i>	0.356 and 0.72 mg/kg/day s.c. for the first and second week, respectively	Human MCF-7 breast cancer cell xenograft in athymic nude mice	Inhibition of tumor growth and angiogenesis; and inhibition of morphine-induced tumor growth and angiogenesis	Gupta et al. (2002)
	100 nmol/L	Human MCF-7 breast cancer cell line	Inhibits basal and 17- $\beta$ estradiol-induced proliferation and MAPK/ERK phosphorylation	Farooqui et al. (2006)
	100 nM	Human non-small cell lung cancer cell line, H2009	Naloxone inhibits MS and EGF induced phosphorylation of EGFR, MAPK/ERK, and Akt and cellular proliferation and invasion	Fujioka et al. (2011)
	1 mM for 6 h	Human ovarian cancer cell line, SKOV-3	Naloxone inhibited cell number by 28%	Donahue et al. (2011b)
	1 $\mu$ M for 6 h	Human ovarian cancer cell line, SKOV-3	Naloxone did not affect cell number	Donahue et al. (2011b)
	1 $\mu$ M	Human ovarian cancer cell lines, OVCAR-3, SKOV-3	Naloxone in the absence or presence of 1 $\mu$ M OGF did not affect ovarian cancer cell proliferation	Donahue et al. (2009)
	100 nmol/L	Human MCF-7 breast cancer cell line	Naloxone blocks opioid induced down regulation of MOR	Gach et al. (2008)
	100 nmol/L	Human MCF-7 breast cancer cell line	Naloxone increased MOR mRNA expression by 20% and protein expression by 68%	Gach et al. (2008)
	100 nmol/L	Human MCF-7 breast cancer cell line	Naloxone administration stimulated the complex formation of NF- $\kappa$ B and AP-1 with the MOR promoter	Gach et al. (2008)
	75 mg/kg/day in diet	7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors	Naltrexone inhibited tumor multiplicity by 40% when administered at tumor initiation, 73% at tumor promotion, and 70% when administered during initiation and promotion	Koo et al. (1996)

0.1 mg/kg/i.p.	Human ovarian cancer cell line SKOV-3 was injected in athymic nu/nu mice and tumors nodules on the surface of liver, stomach, spleen and mesentery/intestine were analyzed	Naltrexone reduced tumor nodules and weight by inhibiting proliferation and angiogenesis	Donahue et al. (2011b)
10 <sup>-5</sup> M	Human ovarian cancer cell line, SKOV-3	Short-term (6 h) Naltrexone treatment inhibited but continuous exposure increased cell proliferation. Naltrexone in conjunction with taxol or cisplatin reduced cell proliferation as compared to either treatment alone	Donahue et al. (2011a)
10 <sup>-5</sup> M short term or continuous exposure of cells in vitro	Multiple human cancer cell lines SKOV-3, OVCAR-3, SCC-1, MiaPaCa-2, HCT-1	Inhibition of proliferation by sort-term (6 h) treatment via opioid growth factor receptor	Donahue et al. (2011a)
100 mg given orally every other day	Patients with untreatable metastatic solid tumors	Naltrexone amplifies lymphocytosis induced by IL-2 and melatonin to enhance their immunotherapeutic ability	Lissoni et al. (2002)
10 <sup>-6</sup> M	Various cancer cell lines; CAL 27, MIA PaCa-2, BxPC-3, HT-29, HCT 116, SKOV-3, OVCAR-3, H226, A549, DU 145, PC-3, SK-HEP, Hep G2, Ht-1080, S-ES-1, SW 1088, U-87 MG, U251, SK-N-SH, <b>MDA-MB-231</b> , <b>MCF7</b> , K-562, AGS, U266, MES-SA, Caki-2, Flo-1, SCC-1, UACC903, 1205 LU, KAT-18	Increased cancer cell proliferation	Zagon et al. (2009)

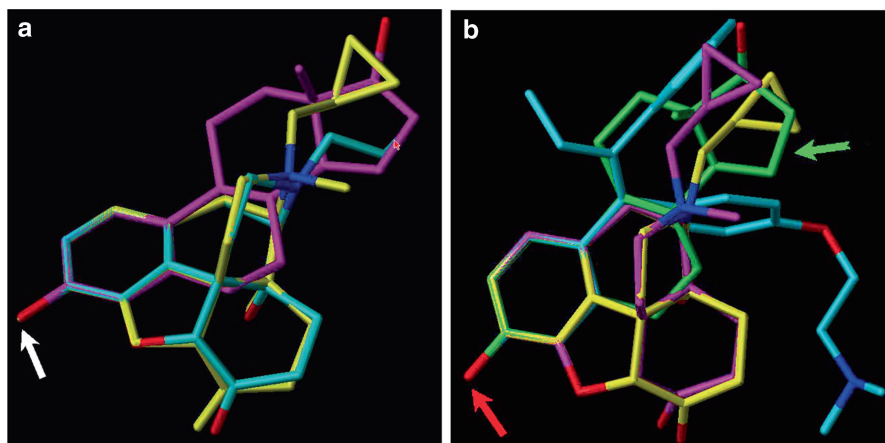
(continued)

Table 2.1 (continued)

Opioid receptor antagonist	Dose/route of administration	Model system	Outcome	Reference
<i>Methylnaltrexone</i>	0.1 mg/kg daily, tri-weekly, or weekly	Human squamous cell carcinoma cell line SCC-1 xenografted in BALB/c athymic nude mice	Increased latency, decreased tumor volume, weight and BrdU incorporation	McLaughlin and Zagon (2012)
	1 $\mu$ M	OVCAR-3, SKOV-3	Naltrexone increased ovarian cancer cell proliferation	Donahue et al. (2009)
	4.5 mg orally at bedtime	Pancreatic cancer with metastasis	Naltrexone given with $\alpha$ -lipoic acid attenuated pancreatic tumor and metastasis	Berkson et al. (2009)
	10 and 100 nM	Lewis Lung Carcinoma (LLC)	Methylnaltrexone significantly reduced LLC cellular invasion	Mathew et al. (2011)
	10 mg/kg/day, s.c.	Xenograft Lewis lung carcinoma r mouse model	Methylnaltrexone significantly reduced tumor volume, tumor weight and lung metastasis	Mathew et al. (2011)
	100 nM	Human pulmonary vein microvascular endothelial cells (HPMVEC)	Methylnaltrexone lowered the IC <sub>50</sub> of 5-FU from 5 $\mu$ mol/L to 7 nmol/L and inhibited Src and Akt activation via MOR	Singleton et al. (2008)
	50 ng/mL	HPMVEC	Methylnaltrexone lowered the IC <sub>50</sub> of bevacizumab from 25 to 6 ng/mL and inhibited Src and Akt activation via MOR	Singleton et al. (2008)
	0.1–500 nM	HPMVEC	Methylnaltrexone alone and in conjunction with mTOR inhibitors reduced VEGF-induced endothelial proliferation and angiogenesis	Singleton et al. (2010)
	100 nM	C57BL6 Mice	Methylnaltrexone alone and in conjunction with mTOR inhibitors reduced angiogenesis	Singleton et al. (2010)
	0.1 $\mu$ M	Human dermal microvascular endothelial cells	Inhibition of VEGF-induced migration, angiogenesis and RhoA activation	Singleton et al. (2006)

Abbreviations used: *MOR* mu opioid receptor, *LLC* Lewis lung carcinoma, *HPMVEC* Human pulmonary vein microvascular endothelial cells, *i.p.* intraperitoneally, *s.c.* subcutaneously





**Fig. 2.1** Structural similarities between estrogen and opioid receptor antagonists, naloxone, naltrexone and methylnaltrexone. (a) Superimposition of energy-minimized structures of E2 (magenta), Nal (cyan), and MNTX (yellow). (b) Superimposition of energy-minimized conformations of E2 (green), NTX (yellow), MNTX (magenta) and 4-hydroxytamoxifen (cyan)

## 2.7 Inhibition of Breast Cancer Growth by Naloxone

Nal at 1.5 mg/kg/day and 10–30 mg/kg/day reduces tumor volume by ~25–30% in nude mice xenografted with MCF-7 human breast cancer cells, compared to controls (Gupta et al. 2002; Tegeder et al. 2003). Nal also antagonizes the genomic and non-genomic activity of ER $\alpha$  in MCF-7 cells (Farooqui et al. 2006). It is important to antagonize ER $\alpha$  activity since most human breast tumors are ER $\alpha$ -positive and respond to estrogen/hormonal therapy, but often develop resistance to therapy. Due to structural similarities with ER $\alpha$  agonists and antagonists, Nal binds to ER $\alpha$  and modulates its activity directly. Nal inhibits E2-induced MAPK/ERK phosphorylation and MCF7 cell proliferation by 65%. Nal directly inhibits the E2-induced activation of ER $\alpha$  by binding to the nuclear ER $\alpha$ , and inhibits E2-induced down-regulation of ER $\alpha$  mRNA, required for receptor re-activation. Moreover, Nal inhibits the non-genomic activity of ER $\alpha$  by inhibiting the binding of E2 to the plasma membrane. In the presence of Nal, ER $\alpha$  associates with MOR only when activated with E2, suggesting the possibility of MOR-induced transactivation of ER (Farooqui et al. 2006).

Nal and NTX are structurally similar OR antagonists that non-selectively bind all three classical ORs and antagonize the analgesic activity of opioids. NTX inhibited neuroblastoma growth at low doses (0.1 mg/kg), but stimulated it at high doses (10 mg/kg) in both immunodeficient and immunocompetent mice. NTX (0.1 mg/kg) increased tumor latency to 98%, increased survival by 36%, but also blocked morphine-induced analgesia for 4–6 h compared to controls (Zagon and McLaughlin 1983a, b, 1987). In contrast, 10 mg/kg NTX had the opposite effect on tumor incidence, latency, survival and metastasis, and blocked morphine-induced analgesia for 24 h. NTX (75 mg/kg diet) also inhibited the initiation (I), progression (P),

and I+P phase of DMBA-induced rat mammary tumors by 27, 60, and 45%, respectively (Koo et al. 1996) and reduced tumor multiplicity by 40, 73, and 70%, respectively. These effects of Nal and NTX support the hypothesis that OR antagonists structurally similar to ER $\alpha$  could be used to treat and inhibit human cancer growth.

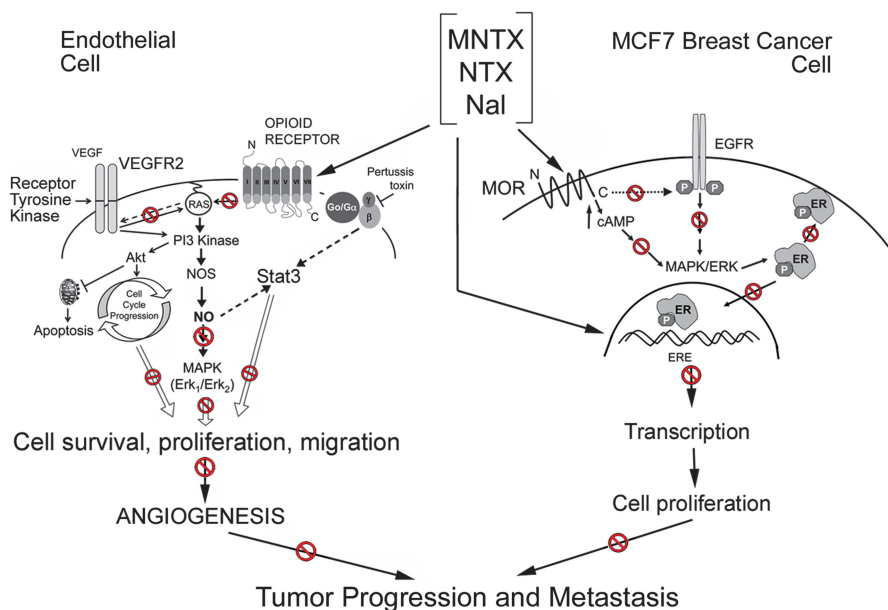
Another OR antagonist, MNTX is a quaternary derivative of NTX, with a methyl group attached to the amine of NTX, has a greater polarity, lower lipid solubility and structural similarity to Nal as described above (Moss and Rosow 2008). Unlike Nal and NTX, MNTX is MOR-selective and does not cross the blood–brain barrier. Therefore MNTX does not antagonize opioid analgesia. MNTX inhibits MOR-mediated vascular endothelial growth factor receptor 2 (VEGFR2) crosstalk, potentiates the apoptotic effect of mTOR inhibitors, inhibits angiogenesis and inhibits lung cancer progression and metastasis (Lennon et al. 2012; Mathew et al. 2011; Singleton et al. 2006, 2010). Table 2.1 lists the effects of OR antagonists on a variety of human endothelial and cancer cells, rodent models of cancer and human cancer. Collectively, the antagonism of MOR and inhibition of cancer growth and angiogenesis by the structurally similar OR antagonists argue for their potential in cancer therapeutics.

## **2.8 OR Antagonism May Uncouple Growth Factor Receptor Signaling Pathways**

MOR also transactivates EGFR (Belcheva et al. 2001; Fujioka et al. 2011). Morphine stimulates MAPK/ERK phosphorylation in endothelial, breast cancer and lung cancer cells via MOR (Singleton et al. 2006; Gupta et al. 2002; Chen et al. 2006; Mathew et al. 2011; Fujioka et al. 2011). Activation of MAPK/ERK and EGFR activates ER $\alpha$  and may even confer tamoxifen (TAM) resistance (Gururaj et al. 2006; Kato et al. 1995; Ring and Dowsett 2004). Higher levels of met-enkephalin were observed in the plasma of women with breast cancer compared to age-matched controls ( $171 \pm 190$  in cancer vs.  $109 \pm 79$  in controls) (Kajdaniuk et al. 2000). Increased endogenous opioids may lead to constitutive activation of MOR, which may contribute to the activation of ER $\alpha$  signaling and perhaps ineffectiveness of ER $\alpha$  based hormonal therapy. It is therefore likely that co-administration of Nal and Nal-like antagonists of ORs may increase the therapeutic efficacy of hormonal therapy.

## **2.9 Translational Significance of Naloxone/OR Antagonism in Cancer Therapy**

Recent studies demonstrate that OR antagonists such as MNTX, which is structurally similar to Nal can inhibit both endothelial and tumor cell proliferation. Therefore, we propose that Nal and Nal-like OR antagonists may attenuate tumor growth by inhibiting



**Fig. 2.2** Proposed model of opioid receptor antagonist(s)-mediated inhibition of breast cancer growth and metastases. Mu opioid receptor transactivates VEGFR2 in endothelium and EGFR and ER $\alpha$  in breast cancer cells. In addition, MOR directly activates angiogenic and growth promoting signaling by activating MAPK/ERK and Stat3 pathway in endothelium. Opioid receptor antagonists, naloxone, naltrexone and methylnaltrexone can inhibit these mitogenic activities of MOR and block cell proliferation. These antagonists can also inhibit the genomic and non-genomic activity of ER $\alpha$  and attenuate estrogen-induced breast cancer cell proliferation (Abbreviations: VEGF vascular endothelial cell growth factor, VEGFR<sub>2</sub> VEGF receptor<sub>2</sub>/Flk1/KDR, MNTX methylnaltrexone, NTX naltrexone, Nal, naloxone; MOR mu opioid receptor, EGFR epidermal growth factor receptor, NO nitric oxide, ER estrogen receptor, ERE estrogen response element)

pro-angiogenic signaling in endothelium; and by inhibiting estrogen-dependent and -independent breast cancer cell proliferation (Fig. 2.2). Nal/NTX/MNTX will block the OR-mediated transactivation of VEGFR2 signaling that is critical to the promotion of angiogenesis. In breast cancer cells, Nal/NTX/MNTX may inhibit (stop signs) the MOR-dependent EGFR and MAPK/ERK signaling which orchestrates resistance to hormonal therapy, as well as directly antagonizes ER $\alpha$  activity, thereby preventing resistance to therapy and inhibiting breast cancer progression. Since metastasis is dependent upon angiogenesis, the anti-angiogenic effect of OR antagonists may even impair metastases. Together, the OR-dependent and independent effect of Nal in breast cancer, support the development of novel Nal-like drugs for cancer therapy.

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