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Opioid Receptors and Ligands: Targets for Cancer Imaging and Therapy

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Abstract

The roles played by classical and non-classical opioid receptors and their ligands in cancer are reviewed, starting with palliation of pain as the traditional linkage, then summarizing current research topics and clinical trials in cancer imaging and therapy, and concluding with a perspective on future directions for the field.

Abbreviations: Akt: Acutely Transforming Retrovirus AKT8 in Rodent T-cell Lymphoma; mTOR: mammalian Target of Rapamycin; EGFR: Epidermal Growth Factor Receptor; OGFr : Opioid Growth Factor Receptor; LDN: Low Dose Naltrexone; ER: Estrogen Receptor; MAPK / Erk: Mitogen-Activated Protein Kinases / Extracellular Signal-Regulated Kinases; SCLC: Small Cell Lung Cancer; PET: Positron Emission Tomography; SPECT: Single Photon Emission Computed Tomography; DO3A: 1 :4 :7 :10-tetraazacyclododecane-1 :4 :7-triacetic acid; BATTLE: Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination; I-SPY TRIAL: Investigation of Serial Studies to Predict your Therapeutic Response with Imaging and Molecular Analysis; ORL: Opioid Receptor-Like

Pain Relief: Traditional Link Between the Opioid System and Cancer

Morphine, the famous natural product of the opium poppy, was termed "God's own medicine" by renowned physician Sir William Osler [1]. Morphine and related opioid analgesics, such as codeine and oxycodone, are first-line therapy for pain. As a consequence, the opioid system is inseparable from palliative care in clinical oncology, and there are nearly 10,000 literature citations to the use of opioid analgesics for control of cancer pain. Modern pain management with opioids that follows the World Health Organization analgesic ladder is safe and effective [2]. Despite little risk of dependency when opioids are used under such clinical guidelines, an unfortunate tendency persists toward under-treatment of cancer pain that adversely affects quality of life [2,3].

Opioid analgesic actions are mediated by three classical G-protein coupled receptors, denoted mu (μ), delta (δ) and kappa (κ), which are located in the brain, spinal cord and certain peripheral organs [4,5]. In the 1970's, the receptors were identified by radioligand binding techniques, and the endorphin, enkephalin and dynorphin peptides were identified as prominent endogenous ligands [6]. In 1997, highly selective m-peptides, termed endomorphins, were added to the roster of endogenous ligands [7-9]. Interestingly, human cells also synthesize morphine *de novo* [10,11]. PET imaging of μ opioid receptors in brain was accomplished in 1984 at Johns Hopkins University using the potent agonist [^{11}C]carfentanil with Professor Henry Wagner as the first volunteer [12,13]. Several reviews have chronicled the development of m, d and k opioid receptor-binding radiotracers for imaging, and their use in clinical studies of drug abuse, neurological disorders and pain [14-16]. Opioid receptors do not necessarily function independently, and can exist as dimers and heterodimers which modulates their pharmacology, and presents new opportunities for drug development

[17,18]. Crystal structures of the μ, δ and κ opioid receptors bound to prototypical antagonist ligands were reported this year in an outstanding series of articles in the journal *Nature* [19-21]. This new knowledge should aid in the development of advanced therapeutics.

The analgesia welcomed by cancer patients is primarily mediated by central μ opioid receptors [22], although peripheral opioid receptors also play key roles [23]. In fact, the development of peripherally restricted opioid analgesics that circumvent centrally mediated side effects, such as respiratory depression, euphoria and mental clouding, is a topic of much current interest [24-26]. Fine-tuning of therapeutic actions is essential, since opioid receptors and their ligands influence a host of physiologic processes including immune, cardiovascular and respiratory functions, feeding behaviors, and smooth muscle contraction [4,5,27]. For instance, the centrally active μ receptor agonists used for relief of chronic cancer pain also activate gastrointestinal opioid receptors, leading to smooth muscle relaxation and constipation. A clinical paradigm for managing bowel dysfunction without affecting analgesia includes parenteral administration of the quaternary salt *N*-methylnaltrexone bromide (Relistor®), an opioid receptor antagonist that is restricted to the periphery [28].

Classical Opioid Receptors: Molecularly Targeted Imaging and Therapy of Cancer

Classical opioid receptors, particularly the μ and δ types, play important direct roles in cell growth and cancer biology that are moving into the spotlight. Historical perspectives and status updates are given below using breast cancer and lung cancer as illustrations.

Breast cancer

Over 200,000 new cases of breast cancer are expected for 2012 in the United States alone [29]. Almost thirty-five years ago, the universal μ, δ and κ opioid receptor antagonists, naloxone and naltrexone, were shown to inhibit the growth of chemically-induced mammary tumors *in vivo* in rats, with the beneficial effects attributed to central inhibition of hormonal secretions required for breast cancer

growth [30]. Radioligand binding studies confirmed that opioid receptors were present on ER-positive breast cancer cells [31,32], and immunohistochemistry studies showed that the vast majority of invasive ductal carcinomas were positive for the endogenous opioid peptides b-endorphin and [Met⁵]-enkephalin [33]. About twenty-five years ago, Zagon and colleagues demonstrated that classical opioid receptors were over-expressed in biopsy specimens from several human cancers, including two breast carcinomas [34]. We now know that these receptors are present with sufficient density to allow clinical imaging, and a non-invasive PET study visualized μ and d opioid receptors on the primary tumors of a breast cancer patient in 2002 [35]. The μ receptor agonist, [¹¹C]carfentanil [12], and the d receptor antagonist, [¹¹C]methylnaltrindole [36,37], were used as the radioligands (Figure 2). Just this year, an epidemiological investigation of 2,039 breast cancer patients correlated stage at presentation, as well as ten-year survival, with A118G polymorphism of the μ opioid receptor gene [38]. This provides strong evidence for critical involvement of the opioid system in breast cancer progression.

Opioid receptors and ligands work through complex mechanisms to modulate cell growth and death [39,40], and the effects of systemic morphine on tumor growth remain controversial to this day [41,42]. On the other hand, increasing brain levels of the endogenous agonist β-endorphin, by transplanting β-endorphin producing neurons into the hypothalamus of rats, causes reduction of chemically-induced mammary tumor incidence, growth and metastasis [43]. Elevated levels of peripheral natural killer cells, macrophage activity and anti-inflammatory cytokines were observed. The anti-cancer effects and stimulation of the immune system were naloxone-reversible, indicating an opioid receptor-mediated process. Somewhat paradoxically, laboratory findings consistently indicate that systemic administration of μ opioid receptor antagonists might be useful for breast cancer therapy [44]. Postulated mechanisms include promotion of ER / μ opioid receptor cross-talk, inhibition of MAPK / Erk phosphorylation, and down-regulation of nuclear ER activity [45]. At present, patients are being recruited for a Phase II clinical trial designed to assess the efficacy of naltrexone against hormone-refractory metastatic breast cancer [46].

Lung cancer

Opioid receptors and ligands are under active investigation as molecular targets for imaging and therapy of lung cancer, the leading cause of cancer deaths worldwide [47]. High expression of classical opioid receptors is a feature of many human lung cancers, but not normal lung tissue. Opioid receptors were detected in cell lines established from primary and metastatic sites of SCLC and non-SCLC over twenty years ago [48,49], and the concept of opioid receptors as targets for diagnostic imaging of lung cancer was espoused about fifteen years ago [50,51]. However, the first PET studies of μ and d opioid receptors in lung cancer patients were reported only five years ago [52]. We used the μ agonist, [¹¹C]carfentanil, and the d antagonist, [¹¹C]methylnaltrindole (Figure 2), to visualize opioid receptors on the primary pulmonary tumors of six SCLC and non-SCLC patients. Uptake of both radioligands was significantly greater in tumors than in normal lung, [¹¹C]methylnaltrindole uptake (δ) was greater than [¹¹C] carfentanil uptake (μ), and tumor receptor binding was blocked in both cases by the universal opioid receptor antagonist naloxone.

These two lipophilic radioligands were developed for brain PET, and are not optimal for peripheral imaging in oncology [14]. A new generation of hydrophilic opioid receptor radioligands, specifically designed for peripheral studies, is now under development. For instance, indium-111 labeled DO3A conjugates of naltrindole (Figure 2) are metabolically stable, maintain very high affinity and selectivity for d opioid receptors *in vitro* [53], and bind tenaciously *in vivo* to d opioid receptors expressed by SCLC tumor xenografts in mouse models [54]. Indium-111 labeled ligands are intended for SPECT imaging, but extrapolation to include special-purpose radiometals having a range of nuclear properties for PET imaging (Cu-64) or molecularly targeted radiotherapy (Lu-177, Y-90) should be possible. Conjugation of a cyanine dye to the d opioid receptor peptide Dmt-Tic-Lys has been reported to yield a fluorescent analog suited for complementary *in vivo* optical imaging studies [55]. So far, attempts to structurally modify μ opioid peptides, including the endomorphins, with radiometal-labeled substituents for *in vivo* imaging have not been successful [56].

Silencing either μ or δ opioid receptors in lung adenocarcinoma cells inhibits EGFR-induced signaling [57], suggesting the possibility of opioid receptor-based lung cancer therapies. Recent and compelling evidence shows that μ opioid receptors, in particular, are integral for the regulation of non-SCLC growth. The μ opioid receptor is over-expressed by 13 of 13 non-SCLC cell lines representing all major histological types, and by 30 biopsy specimens of human non-SCLC tumors with respect to adjacent normal lung [58,59]. Further, silencing μ receptors, or continuous antagonist blockade with naltrexone or N-methylnaltrexone bromide, inhibits non-SCLC growth and metastasis *in vivo* in animal models [58,59]. Moreover, Lewis lung carcinoma cells did not form syngeneic tumors when given to μ opioid receptor knockout mice [58]. Transfection of a bronchoalveolar carcinoma cell line to induce even greater over-expression of μ opioid receptors also augmented its flank tumor growth rate and level of lung metastasis
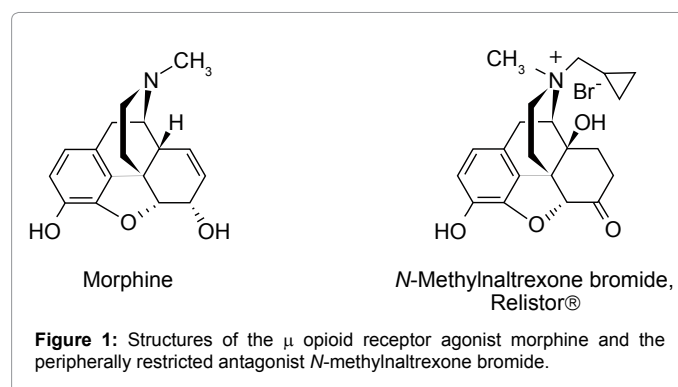

Figure 1: Structures of the μ opioid receptor agonist morphine and the peripherally restricted antagonist N-methylnaltrexone bromide.
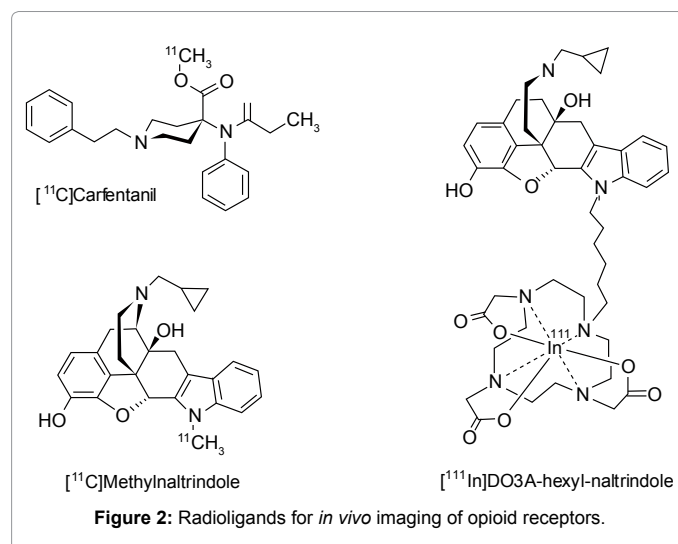

Figure 2: Radioligands for *in vivo* imaging of opioid receptors.

in nude mice [59]. Activation of serine / threonine kinase pathways, Akt and mTOR, are the primary mechanisms identified for μ opioid receptor mediation of non-SCLC progression [59]. These laboratory studies were driven, in part, by observations made at the University of Chicago that certain cancer patients receiving *N*-methylnaltrexone bromide for relief of μ opioid-induced constipation exhibited longer than anticipated survival [60]. Thus, *N*-methylnaltrexone bromide (Figure 1) is a promising candidate for repurposing as a lung cancer therapeutic.

Opioid Growth Factor and Receptor for Targeted Therapy of Cancer

In 1989, Zagon, McLaughlin and colleagues at Pennsylvania State University reported the discovery of a distinct, non-classical binding site for opioids that they initially termed the zeta (ζ) opioid receptor [61]. Further pioneering work showed the site to be a membrane protein, associated with the nucleus, which bears no structural resemblance to classical opioid receptors located on the cell surface [62]. This site has been renamed the opioid growth factor receptor (OGFr). [Met^5]-enkephalin (Figure 3) has been termed the opioid growth factor (OGF) because of potent inhibition of cancer cell growth through OGFr *in vitro* when other opioid peptides, such as b-endorphin, were without effect [62]. Activation of OGFr modulates DNA synthesis, and appears to inhibit cell growth by translocation of peptide-receptor complexes adjacent to heterochromatin inside the nucleus [62].

Traditional opioid receptor antagonists, such as naltrexone, also bind to OGFr and can block the actions of [Met^5]-enkephalin. In turn, [Met^5]-enkephalin also binds well to the classical δ and μ opioid receptors. Thus, differentiation of the opioid receptor(s) responsible for the various actions of [Met^5]-enkephalin and naltrexone can be difficult. In the early 1980's, before the discovery of the OGFr, studies by Zagon and McLaughlin [63,64] showed that naltrexone modulates neuroblastoma tumors in mice in strikingly dose-dependent fashion. Daily treatments with naltrexone at 0.1 mg / kg blocked opioid receptors for 6 - 8 hours per day, reduced tumor incidence to 33%, delayed tumor appearance by 98%, and increased survival time by 36%. By contrast, daily treatments with naltrexone at 10.0 mg / kg blocked opioid receptors for a full 24 hours per day, gave a tumor incidence of 100%, delayed tumor appearance by 27%, and decreased survival time by 19%. Both naltrexone regimens caused up-regulation of classical opioid receptor sites on the tumors, and increased tissue levels of β-endorphin and [Met^5]-enkephalin by up to six-fold. However, only the chronic low dose naltrexone (LDN) protocol gave significant antitumor effects. Zagon and McLaughlin surmised that endogenous opioids are trophic agents that inhibit tumor growth by suppressing cell proliferation, and that the duration of opioid receptor blockade

by antagonists is critical for modulation of these actions *in vivo*. Thus, intermittent blockade up-regulates opioid peptides and receptors, leading to decreased tumor cell growth during those times when the antagonist is absent [65].

In 2011, Donahue et al. [66] used an elegant tissue culture model system to show that exposure of cancer cells to naltrexone for a short period of time inhibits their growth, and that up-regulation of OGFr - OGF appears to be uniquely responsible. Ovarian cancer, pancreatic cancer, squamous cell carcinoma of the head and neck, and colorectal cancer cell lines all gave similar results. In companion studies, OGF and LDN were shown to significantly inhibit the progression of human ovarian cancer in a nude mouse model [67], an effect that could be further enhanced by combination with cisplatin, a standard of care chemotherapeutic agent [68]. As discussed in detail by Donahue et al. [66-68], the findings provide mechanistic support for the use of OGF and LDN in cancer therapy. The primary component of their current LDN concept is that repetitive, but short-term, blockade of OGFr with naltrexone increases OGFr number as well as levels of OGF. As naltrexone is eliminated from the body, amplified OGFr and OGF are able to interact again with increased ability to inhibit cancer cell proliferation.

The efficacy of OGF alone for treatment of advanced pancreatic cancer was demonstrated in a recent Phase II clinical trial involving twenty-four patients who had failed standard of care chemotherapy [69]. Weekly treatment with intravenous OGF led to 3-fold longer survival time as compared to untreated patients, and 62% of subjects who survived more than two months had decreased or stabilized tumor mass. A more limited cohort of four patients with advanced pancreatic cancer exhibited much longer than expected survival in response to a combination of LDN and a-lipoic acid, an enzyme cofactor with antioxidant properties and beneficial effects on immune cell function [70]. In a case report, six months of LDN therapy alone led to significant improvements in lymph node size and metabolic activity for a single patient with B-cell lymphoma [71]. Naltrexone exerts neuroimmunomodulatory effects itself, albeit at higher dosage levels. Lissoni et al. [72,73] documented naltrexone suppression of T helper-2 cell activity and amplification of the anti-cancer cytokine, interleukin 2, in clinical trials involving cancer patients with diverse metastatic solid tumors. Two upcoming clinical trials posted at clinicaltrials.gov will test LDN against metastatic melanoma, castration-resistant prostate cancer and renal cancer [74], and as an agent for improving quality of life in glioma patients [75]. Thus, the LDN concept is gaining traction within the mainstream medical community. LDN is often mentioned on the Internet [76,77] and in the popular press [78] as a promising, inexpensive off-label adjuvant cancer treatment, replete with anecdotal descriptions of beneficial effects.

Perspectives

The remarkable ability of opioid receptors and opioid analgesics to alleviate pain is their traditional link to cancer, a union that will no doubt continue. However, the relationship is becoming much more meaningful. In their 1986 article in the journal *Cancer*, Roth and Barchas [48] reported the presence of opioid peptides and receptors on SCLC cells, and presciently stated: "If individual cancers can be defined by the peptides and receptors they express, it may be possible to design rational therapy in an individualized manner." We are fortunate to be in an era where personalized medicine is becoming a reality. Although a challenging proposition, innovative, molecularly targeted therapies for cancer can be matched to the particular patients most likely to benefit [79-81]. The BATTLE trial in lung cancer [82] and the I-SPY TRIAL in breast cancer [83] are major steps forward, showing the feasibility of
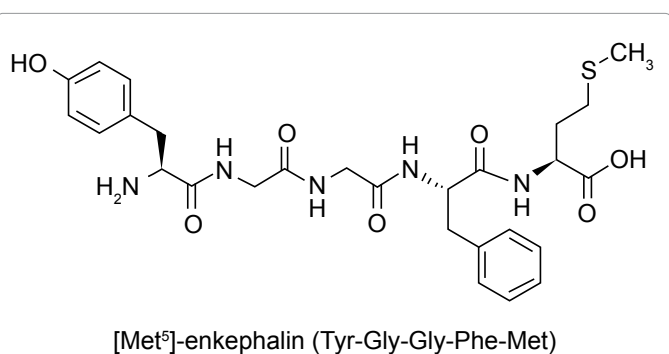

Figure 3: Structure of [Met^5]-enkephalin, a major endogenous opioid growth factor.

using a patient's own biomarkers to select the best treatment option for them. To note but one important example, non-SCLC patients having EGFR mutations are a small percentage of the total patient population, but are very responsive to tyrosine kinase inhibitors such as erlotinib (Tarceva®), leading to notable survival benefits [84].

Over the past several years, significant progress has been made in identifying the roles that classical and non-classical opioid receptors and their ligands play in cancer biology, and the pace of discovery has quickened. Some mysteries, and many questions, remain. For instance, relatively little is known about direct involvement of the k opioid receptor in cancer. More information would be welcome, since a study this year showed that a selective k agonist inhibits the growth of non-SCLC cells, in naloxone-reversible fashion, through a mechanism involving death-promoting glycogen synthase kinase 3b [85]. Scant information is available relating the fourth recognized opioid receptor, ORL$_1$, to cancer. ORL$_1$, also known as the nociceptin / orphanin FQ receptor, is an intriguing G-protein-coupled site that displays about 60% sequence homology to the classical opioid receptors, but does not bind most traditional opioids because of conformational differences in its ligand binding pockets [86,87]. ORL$_1$ activation may cause inhibition of pro-inflammatory cytokines and chemokines [88], but more specific connections to cancer remain to be established. An emerging topic for exploration is modulation of μ and d opioid receptor homo- and heterodimerization *in vivo*, a phenomenon which influences mammary tumor growth in a rat model by an unknown mechanism [89].

Abundant evidence shows that cancer therapy mediated by opioid receptors and their ligands is a real possibility. Phase II clinical trials with OGF, as well as high- and low-dose naltrexone, are already being conducted with some success. The anticancer effects to be gained from continuous opioid receptor blockade, as discussed above for breast and lung cancer therapy in animal models, would not be likely under the mechanistic paradigm of LDN alone. On the other hand, LDN clearly is effective in some animal models when continuous receptor blockade is not. Taken together, the available laboratory data point to multiple mechanisms for opioid actions on cancer cell growth that involve the classical opioid receptors, as well as the putative non-classical OGFr that has not been as widely studied. The effects observed are often dependent upon study specifics. Thus, routine clinical integration of opioid-mediated cancer therapy will require a much better understanding of the complex interplay between the various molecular mechanisms that may be involved, as well as concrete results from well-structured clinical research trials.

Molecular imaging is sure to play a part in the continued translation of the research into the clinic. Taking the personalized medicine approach, one might envision μ opioid receptor PET as a way to stratify lung cancer patients into a cohort that would benefit from μ receptor antagonist therapy. Perhaps patients with cancers that over-express d opioid receptors could be identified by SPECT using a ligand labeled with the g-emitter In-111, and then treated by targeted radiotherapy using an analog labeled with a cell-killing b-emitter such as Y-90. In addition to their complex effects on cancer cell growth, opioid receptors and their ligands also mediate many normal functions. Thus, the therapeutic "window" for opioids in oncology might be narrow, but is definitely worth opening.

Acknowledgements

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