

# THE MU OPIATE RECEPTOR AS A POTENTIAL THERAPEUTIC TARGET IN LUNG CANCER

Biji Mathew, Ph.D.<sup>1</sup>, Frances E Lennon, Ph.D.<sup>2</sup>, Jonathan Moss, M.D., Ph.D.<sup>3</sup>, Patrick A Singleton, Ph.D.<sup>2</sup>

<sup>1</sup>Pulmonary, Critical Care, and Sleep Medicine, University of Illinois, Chicago, Illinois

<sup>2</sup>Department of Medicine, The University of Chicago, Chicago, Illinois

<sup>3</sup>Department of Anesthesia and Critical Care, The University of Chicago, Chicago, Illinois

**Introduction:** Recent epidemiologic studies suggest that tumor recurrence may be related to the type of anesthesia employed (Exadaktylos AK et al., *Anesthesiology* 2006; Biki B et al., *Anesthesiology* 2008). Previous work by our laboratory and others suggested a role of opioids in several aspects of tumor growth (Wang et al., *Anticancer Res*, 2009; Singleton et al., *Mol Cancer Ther*, 2008; Gupta et al., *Cancer Res*, 2002). Expression of the mu opioid receptor (MOR) is increased in patients with non-small cell lung cancer (NSCLC), a disease with poor prognosis and limited therapies. In this study, we investigated MOR as a potential therapeutic target for lung carcinoma.

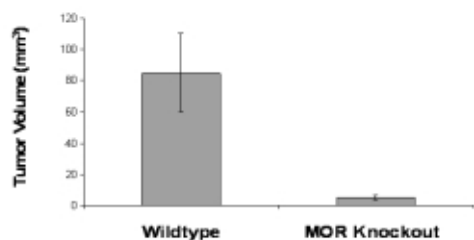
**Methods:** Lewis lung carcinoma (LLC) cells were treated with MOR siRNA or the peripheral MOR antagonist, methylaltraxone (MNTX, 250 nM), prior to the addition of EGF (10 ng/ml), IGF (10 ng/ml), DAMGO (1 nM), morphine (1 nM) or serum (1, 5 or 10%). In vitro functional (cell proliferation and invasion) and biochemical studies (immunoblotting) were then conducted. For in vivo assays, C57BL/6J mice were injected intravenously with dual color (GFP-RFP labeled) LLC cells, with or without MOR siRNA treatment. In another experiment, MOR knockout as well as wildtype control mice were injected with LLC cells (subcutaneously into the flank) and followed for 12 days. In a parallel experiment, LLC tumor bearing C57BL/6J mice received continuous infusion of MNTX (10 mg/kg/day) for 9 days. Tumor growth and lung metastasis were evaluated using tumor volume measurements and/or in vivo fluorescent microscopy (Olympus OV-100) with or without intravenous injection of ProSense and/or MMP Sense probes. In vivo angiogenesis was measured with subcutaneously implanted Matrigel plugs containing 20 ng VEGF with or without 100 nM MNTX. After 21 days, the plugs were removed and analyzed for hemoglobin content.

**Results:** Our data indicate that LLC cells have 5 fold increased expression of MOR (compared to primary lung epithelial or BEAS-2B cells). Inhibition of MOR with siRNA or MNTX treatment reduced in vitro LLC proliferation (90%) and invasion (50-75%). In vivo lung metastasis was reduced by 75% in wildtype C57BL/6J mice with LLC lacking MOR (siRNA) versus control siRNA-treated LLC (3 weeks post-i.v. injection, quantitated with OV-100). In addition, primary LLC tumor volume was substantially reduced (>90%) in MOR knockout mice compared to wildtype mice (flank injection).

We observed that continuous infusion of MNTX (9 days, 10 mg/kg/day) in mice with preexisting LLC primary tumors attenuates further tumor growth by 43%. Finally, Matrigel plugs with MNTX had 45% less in vivo angiogenesis versus control (no MNTX) Matrigel plugs.

**Conclusion:** Our data demonstrate that MOR is a potential therapeutic target for inhibition of LLC proliferation, invasion and angiogenesis and provide a plausible explanation for the epidemiologic observations on anesthetic technique and cancer recurrence.

## Inhibition of tumor formation in the mu opioid receptor knockout mouse



MOR-1 knockout mice and appropriate WT controls were injected (SQ) with LLC(  $1 \times 10^6$ ) in right flank. Tumor growth was measured after 12 days. n=6