

# Cyclooxygenase activity and tumor progression

## AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet kommer offentligen att försvaras i Aulan, Centralkomplexet, Sahlgrenska Universitetssjukhuset, Göteborg, fredagen den 3 oktober 2008 kl 09.00

av

Christian Cahlin  
Leg.läkare

Fakultetsopponent:  
Docent Henrik Thorlaciuss  
Kirurgiska kliniken, Universitetssjukhuset MAS, Malmö

Avhandlingen baseras på följande delarbeten:

- I Lönnroth C, Svaninger G, Gelin J, Cahlin C, Iresjö BM, Cvetkovska E, Edström S, Andersson M, Svanberg E, Lundholm K. Effects related to indomethacin prolonged survival and decreased tumor growth in a mouse tumor model with cytokine dependent cancer cachexia. *Int J Oncol.* 7: 1405-1413, 1995.
- II Cahlin C, Gelin J, Delbro D, Lönnroth C, Doi C, Lundholm K. Effect of cyclooxygenase and nitric oxide synthase inhibitors on tumor growth in mouse tumor models with and without cancer cachexia related to prostanoids. *Cancer Research.* 60: 1742-1749, 2000.
- III Cahlin C, Körner A, Axelsson H, Wang W, Lundholm K, Svanberg E. Experimental cancer cachexia: The role of host-derived cytokines Interleukin (IL)-6, IL-12, interferon- $\gamma$ , and tumor necrosis factor  $\alpha$  evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Research.* 60: 5488-5493, 2000.
- IV Cahlin C, Gelin J, Andersson M, Lönnroth C, Lundholm K. The effects of non-selective, preferential-selective and selective COX-inhibitors on the growth of experimental and human tumors in mice related to prostanoids receptors. *Int J Oncol.* 27(4): 913-23, 2005.
- V Cahlin C, Lönnroth C, Arvidsson A, Nordgren S, Lundholm K. Growth associated proteins in tumor cells and stroma related to disease progression of colon cancer accounting for tumor tissue PGE<sub>2</sub> content. *Int J Oncol.* 32: 909-918, 2008.



GÖTEBORGS UNIVERSITET

# Cyclooxygenase activity and tumor progression

Christian Cahlin

Departments of Surgery and Transplantation, Institute of Clinical Sciences  
at Sahlgrenska Academy, University of Gothenburg  
Gothenburg, Sweden, 2008

## Abstract

Invasive growth of malignant tumors is associated with local and systemic inflammation, which may promote progression and metastases. Inflammation is also responsible for appearing manifestations of advanced cancer as fatigue, anorexia and wasting with eicosanoids, proinflammatory cytokines and nitric oxide as mediators. The aim of the present work was to extend information on the significance of cyclooxygenase activity in local and systemic progression of tumor disease.

**Methods:** Murine tumor models (MCG-101, K1735-M2), human carcinomas xenotransplanted to nude mice, tumor cell cultures and tissue samples from human colorectal adenocarcinomas were used. Inhibitors of cyclooxygenase and nitric oxide synthase, antibodies against IL-6 and recombinant IL-12 were used to evaluate effects on tumor growth, inflammation (SAP, CRP, ESR) and host wasting (anorexia, body composition). Expression of proteins was evaluated by immunohistochemistry, western blot and RT-PCR. Signal molecules were quantified by RIA, ELISA and immunoelectrophoresis. Eicosanoids and polyamines were fractionated by HPLC. Cell proliferation was estimated by mitotic counting and flow cytometry.

**Results:** Inhibition of prostaglandin synthesis (indomethacin) reduced tumor growth, attenuated host wasting and prolonged survival in MCG-101 bearing mice with high tumor production of PGE<sub>2</sub>. By contrast, no such effects were seen in K1735-M2 bearing mice with insignificant PGE<sub>2</sub> production. Indomethacin also reduced growth of human tumors on nude mice. There was no clear-cut correlation between overall COX-2 expression in tumors and sensitivity to indomethacin treatment, although COX-2 expression was significantly correlated to tumor PGE<sub>2</sub> production; factors that predicted reduced survival in colon carcinoma. IL-6 deficient mice showed reduced tumor growth and wasting. Indomethacin reduced plasma PGE<sub>2</sub> levels and wasting in all groups of cytokine knockout mice, but only IL-12 knockouts showed concomitant reduction in tumor growth. Recombinant IL-12 reduced tumor growth in wild type mice, but not so in IFN- $\gamma$  deficient mice. Cytokine knockout tumor-bearing mice experienced anorexia to the same extent as wild types. Our results suggested subtype EP receptors to explain effects by PGE<sub>2</sub> exposure to tumor and stroma cells. Systemic inflammation was related to tumor cell proliferation evaluated by p15, TGF $\beta$ 3 and Bcl-2 in tumor tissue. Indomethacin treatment increased tumor tissue expression of IL-6, TNF- $\alpha$ , GM-CSF, TGF $\beta$ , cNOS decreased expression of b-FGF, angiogenin, vWF and blood vessel density, whereas EGF, VEGF, PDGF A, B, IL-1 $\alpha$ , transferrin receptors were unchanged. Cell cycle was prolonged in vivo but not in vitro by indomethacin. NSAID inhibition of tumor growth and host-wasting was not simply related to COX specificity. NOS-inhibitors reduced tumor growth in both MCG-101 and K1735-M2 tumors expressing high amounts of cNOS and iNOS. Synergism between COX- and NOS-inhibition was not observed. NOS inhibition attenuated host wasting to the same extent as indomethacin in MCG-101 bearing mice.

**Conclusion:** Results in the present study demonstrate that cyclooxygenase activity is central in tumor progression with well-recognized host stigmata of systemic inflammation both in experimental and clinical cancer. Such effects are connected to classic tumor growth factors, cytokines and nitric oxide, where redundancy among cytokines was pronounced in development of host deteriorations (cachexia).

**Key words:** cyclooxygenase, PGE<sub>2</sub>, inflammation, cytokine, IL-6, nitric oxide, indomethacin