Physiological Reports

Open Access

Physiological Reports ISSN 2051-817X

ORIGINAL RESEARCH

Food intake, tumor growth, and weight loss in EP_2 receptor subtype knockout mice bearing PGE_2-producing tumors

Britt-Marie Iresjö[1], Wenhua Wang[1], Camilla Nilsberth[2], Marianne Andersson[1], Christina Lönnroth[1] & Ulrika Smedh[1]

1 Surgical Metabolic Research Laboratory, Department of Surgery, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
2 Department of Geriatric Medicine and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Keywords
Anorexia, cachexia, EP receptor, hypothalamus, microarray analysis, Prostaglandin D synthase.

Correspondence
Britt-Marie Iresjö, Surgical Metabolic Research Laboratory, Department of Surgery, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden.
Tel: +46-31-342-3678
E-mail: britt-marie.iresjo@surgery.gu.se

Funding Information
Supported in parts by grants from the Swedish Cancer Society, The Swedish Research Council, Assar Gabrielsson Foundation (AB Volvo), Byggmästare Olle Engkvists stiftelse, IngaBritt & Arne Lundberg Research Foundation, Swedish and Gothenburg Medical Societies and the Medical Faculty, University of Gothenburg.

Received: 17 March 2015; Revised: 30 May 2015; Accepted: 4 June 2015

doi: 10.14814/phy2.12441

Physiol Rep, 3 (7), 2015, e12441, doi: 10.14814/phy2.12441

Abstract

Previous studies in our laboratory have demonstrated that prostaglandin (PG) E_2 is involved in anorexia/cachexia development in MCG 101 tumor-bearing mice. In the present study, we investigate the role of PGE receptor subtype EP_2 in the development of anorexia after MCG 101 implantation in wild-type ($EP_2^{+/+}$) or EP_2-receptor knockout ($EP2^{-/-}$) mice. Our results showed that host absence of EP_2 receptors attenuated tumor growth and development of anorexia in tumor-bearing EP_2 knockout mice compared to tumor-bearing wild-type animals. Microarray profiling of the hypothalamus revealed a relative twofold change in expression of around 35 genes including mRNA transcripts coding for Phospholipase A_2 and Prostaglandin D_2 synthase (*Ptgds*) in EP_2 receptor knockout mice compared to wild-type mice. Prostaglandin D_2 synthase levels were increased significantly in EP_2 receptor knockouts, suggesting that improved food intake may depend on altered balance of prostaglandin production in hypothalamus since PGE_2 and PGD_2 display opposing effects in feeding control.

Introduction

Tumors are known to cause inflammation through release of cascades of inflammatory signals including interleukins and prostaglandins in order to promote growth (Lönnroth et al. 1995). Cytokines and eicosanoides cause a variety of secondary physiological responses of the host, including anorexia and weight loss (Plata-Salaman 1999; Furuyashiki and Narumiya 2011). The precise mechanisms of prostaglandins to alter feeding and metabolism are, however, not fully known. Prostaglandins act on specific EP receptor subtypes which are transmembrane spanning, G-protein coupled receptors classified as EP_1, EP_2, EP_3, and EP_4. Each EP receptor is associated with a unique G-protein and a second messenger system, but signaling can also be transduced by G-protein-independent mechanisms (Jiang and Dingledine 2013a). Previously, attention has been paid to the role of EP receptor subtypes 1, 3, and 4 in anorexia secondary to tumor growth (Wang et al. 2001, 2005a; Ruud et al.

2013). However, since cyclooxygenase inhibition by indomethacin failed to improve food intake but maintained body composition in tumor-bearing animals genetically depleted of EP_1 or EP_3 receptors, it would appear that these receptors do not participate in a prostaglandin-induced anorexic response (Wang et al. 2005a). A possible candidate for central anorexia could be EP_4 receptor since ICV injection of an EP_4 antagonist blocked the anorexic effect of PGE_2 administration in healthy mice (Ohinata et al. 2006). However, PGE_2-EP_4 receptor ligand binding does not seem to be the underlying mechanism in tumor-induced hypophagia since CNS-specific disruption of EP_4 receptors did not alter the anorexic response in MCG 101 tumor-bearing animals (Ruud et al. 2013).

From a clinical perspective, PGE_2 has raised interest since it may be released from epithelial tumors such as colon cancer in progressive disease (Yang et al. 1998; Cahlin et al. 2008). There are several possible mechanisms for PGE_2 to reach its central target receptors. PGE_2 is highly lipophilic and can readily cross the blood–brain barrier but has a very short half-life in the circulation, and passive diffusion has been suggested to be of less importance (Ruud et al. 2013). Instead circulating PGE_2 was suggested to act in the circumventricular organs and induce central PG synthesis and release via COX-activation (Laflamme et al. 1999). Prostaglandins display significant cross-reactivity on all of the four subtypes of EP receptors (Kiriyama et al. 1997) and $EP_{1–4}$ receptors are present in hypothalamus and brainstem areas of relevance for feeding control and metabolism (Zhang and Rivest 1999; Wang et al. 2005b; Ruud et al. 2013).

The aim of the present study was to evaluate the role of subtype EP_2 receptor signaling for development of anorexia in tumor-bearing animals since genetic knockout studies could not verify a role of other PGE receptor candidates as EP_1, EP_3, or EP_4, in mediating the prostaglandin-induced anorexic response of the tumor-bearing host (Wang et al. 2005a; Ruud et al. 2013). For this purpose we used a solid tumor model, MCG 101, which induces anorexia and cachexia in part due to elevated intrinsic production of PGE_2. In order to explore the role of the EP_2 receptor for anorexia development, an $EP_2^{-/-}$ knockout mice model was used.

Materials and Methods

Animal experiments

The animal experimental protocol was approved by the Regional committee for animal ethics in Göteborg. Adult, male and female and age-matched $EP_2^{-/-}$ and $EP_2^{+/+}$ mice (C57BL/6 genetic background) (Tilley et al. 1999) were bred and housed in plastic cages in a temperature controlled room with a 12 h dark/light cycle and received standard laboratory rodent chow (B & K Universal AB, Stockholm, Sweden). Animal groups were tumor-bearing (TB) and sham-treated controls (FF) in $EP_2^{-/-}$ and $EP_2^{+/+}$ mice. All animals had free access to tap water and food at all times before and during experiments. Prior to experiments, mice were transferred to cages with wire floor that permitted collection and quantification of spilled food by weighing. Daily food intake and body weight were registered in the morning between 08.00 and 09.00. Animals were allowed 3 days adaptation to wire floors before the start of experiments (day 0) (Lönnroth et al. 1995; Wang et al. 2005a,c). Tumor-bearing mice were implanted s.c. bilaterally in the flank with a 3–5 mm^3 of a transplantable MCG-101 methylcholanthrene-induced tumor under general anesthesia (Isofluran, inhaled concentration 2.7%) (Lundholm et al. 1978). Control mice were sham implanted. All mice were sacrificed on day 10 upon tumor implantation between 8–11 AM. Blood samples were obtained by cardiac puncture during general anesthesia for plasma PGE_2 determination followed by 20 mL 4°C transcardiac saline perfusion (Lönnroth et al. 1995; Wang et al. 2001). The brains were rapidly removed and hypothalamus was dissected free, snap-frozen in liquid nitrogen, and kept at −80°C until micro-array analyses. Dry tumor weight, water content, fat-free carcass weight, and whole-body fat were determined as described (Eden et al. 1983).

RNA extraction

Total RNA was extracted using RNeasy Lipid Tissue mini kit (Qiagen GmbH, Hilden, Germany) with on column DNase treatment included according to kit protocol. Quality of RNA was checked in an Agilent 2100 BioAnalyzer with the RNA 6000 Nano Assay kit (Agilent Technologies, Inc., Santa Clara, CA). The concentration of RNA was measured in a Nano Drop ND-1000A spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Hypothalamic mRNA for microarray analysis was pooled from seven mice in each group.

Real-time PCR

Two hundred nanograms of total RNA from each hypothalamus were reverse transcribed in a cDNA synthesis reaction using oligo d(T) primers according to the manufacturer's instructions (Advantage® RT for PCR kit; Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France). Positive and negative controls were included in each run of cDNA synthesis. Predesigned primers from Qiagen were used for analysis of mouse *Ptgs1*, (Cox1, Assay 00155330) *Ptgs2*, (Cox2, Assay QT00165347) and

Ptgds (PGD_2 synthase, Assay QT00098049). Real-time PCR analysis was performed using either QantiTect SYBR Green kit or LightCykler FastStart DNA MasterPLUS SYBR Green I kit (Roche Diagnostics Scandinavia AB, Bromma, Sweden). Two microliter of diluted cDNA and 2 μL of primer were used for each reaction of 20 μL. All samples were analyzed in duplicates, and positive and negative results were included in each run. A LightCykler 1.5 instrument was used for all analyses. Quantitative results were produced by the relative standard curve method using GAPDH as housekeeping gene, which was equally expressed among groups.

Microarray expression profiling

Five hundred nanograms of pooled total RNA from each group were labeled with Cyanine 3-dCTP or Cyanine 5-dCTP (GE Healtcare Life sciences, Uppsala, Sweden) in a cDNA synthesis reaction using the Agilent Fluorescent Direct Label Kit (n = 7/group). Whole Mouse Genome Oligo Microarray (4 × 44K; Agilent Technologies) containing 41,174 features, including positive and negative control spots, were used. Hybridization was performed during 18 h with $EP_2^{-/-}$ TB versus $EP_2^{+/+}$ TB cDNA in a dual-color experiment, followed by posthybridization washes according to "in situ Hybridization Kit Plus" (Agilent Technologies) instructions. Two technical replicates were done. The microarrays were dried with nitrogen gas in laminar flow and images were quantified on an Agilent G2565 AA microarray scanner. Fluorescence intensities were extracted using the Feature Extraction software program v9.1.3.1. (Agilent Technologies). Dye-normalized, outlier- and background-subtracted values were imported with the FE Plug-in (Agilent Technologies) into GeneSpring software program v 12.5 that was used for data analysis. Of the 41,232 features on the array, 18,851 features from pooled hypothalamus RNA were detected as present, with a signal ≥2.6 SD above background signal; 1747 entities remained after t-test against zero ($P < 0.05$). Fold changes 1.5 of Log2 transformed ratios were considered statistically significant in gene expression and used for further analyses in Gene Ontology search and pathway analysis. A fold change of 1.5 corresponds to a change in gene expression of 50% which has been reported to generate reproducible sets of altered genes when compared across microarray platforms (Patterson et al. 2006).

Statistics

Results are presented as mean ± SE. Food intake and animal weight over time were compared by two-way ANOVA for repeated measures. End point variables (tumor weight, body composition, plasma PGE_2 concentration and mRNA levels) were compared by one-way factorial ANOVA followed by Fisher PLSD, or t-test when appropriate. $P \leq 0.05$ was considered statistically significant in two-tailed tests. Statview for Windows v. 5.0.1 was used for statistical calculations. Statistical evaluations of microarray analyses were done in Genespring 12.5 software as described in the Materials and Methods section.

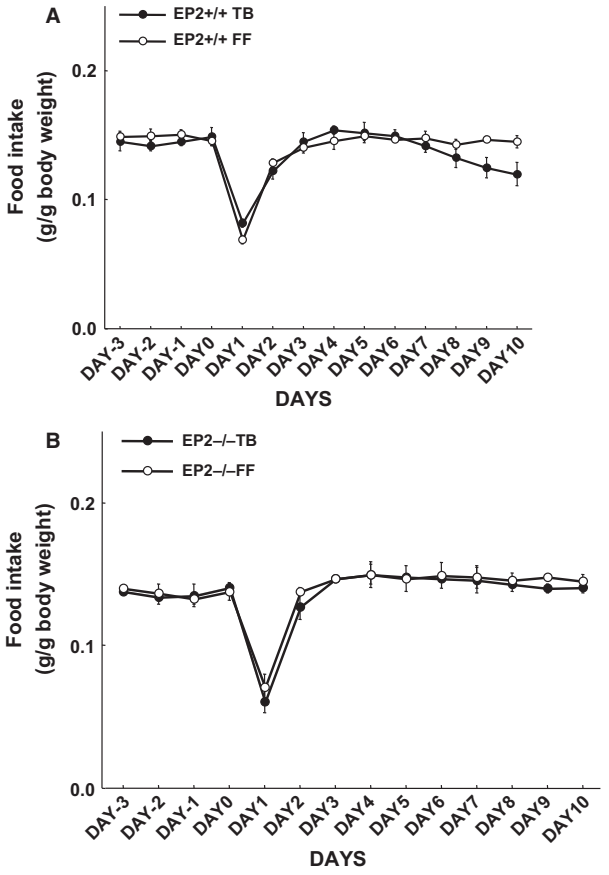

Figure 1. Time-course changes of food intake in $EP_2^{+/+}$ (A) and $EP_2^{-/-}$ (B) tumor-bearing mice (TB) and sham controls (FF). Food intake decreased significantly in TB wild-type mice from days 6 to 7 when tumor mass appeared ($P < 0.05$, A) (mean ± SEM, seven animals in each observation point; ANOVA for repeated measures).

Results

Food intake

Food intake declined significantly in wild-type tumor-bearing mice around day 7 and remained lower compared to sham controls in wild-type $EP_2^{+/+}$ mice (Fig. 1A). There was no significant tumor-induced anorexia in tumor-bearing $EP_2^{-/-}$ knockouts (Fig. 1B).

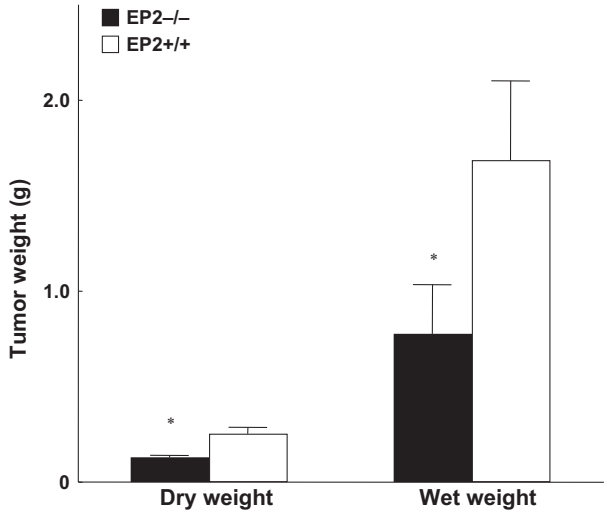

Figure 2. Tumor wet and dry weight at the end of experiments (day 10) in $EP_2^{-/-}$ and $EP_2^{+/+}$ tumor-bearing mice (mean ± SEM, *$P < 0.05$; seven animals in each group).

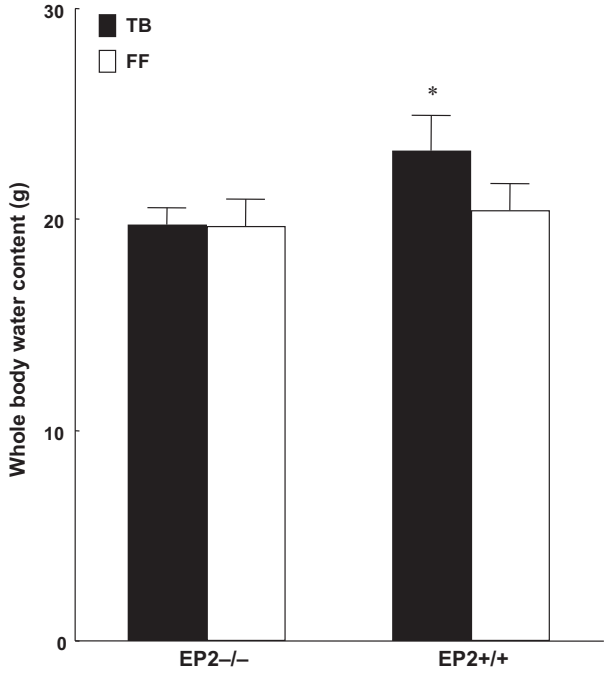

Figure 3. Whole-body water content in freely fed tumor-bearing mice (TB) and sham controls (FF) at the end of experiment (day 10) (mean ± SEM, *$P < 0.05$; seven animals in each group).

Tumor weight and body composition

Tumor wet and dry weight were significantly lower at the end of the experiment in knockout mice ($EP_2^{-/-}$) compared to wild-type animals ($EP_2^{+/+}$) ($P < 0.05$; Fig. 2).

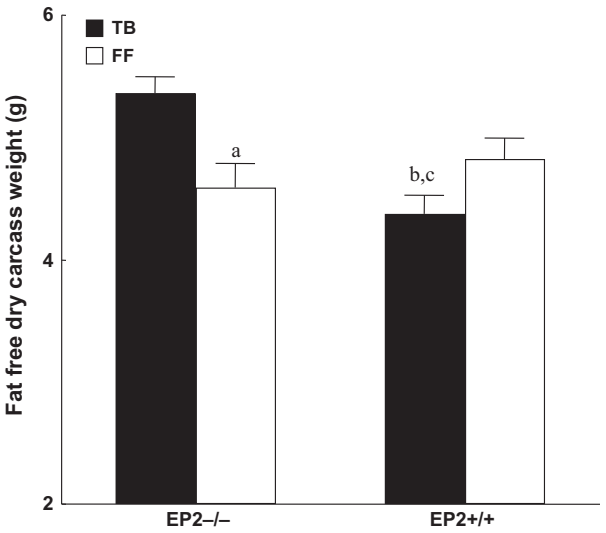

Figure 4. Whole-body fat-free carcass dry weight at the end of the experiments (day 10) in freely fed tumor-bearing animals (TB) and sham controls (FF) (mean ± SEM, (a) $P < 0.01$ versus TB $EP_2^{-/-}$; (b) $P < 0.07$ versus FF $EP_2^{+/+}$; (c) $P < 0.001$ versus TB $EP_2^{-/-}$; seven animals in each group).

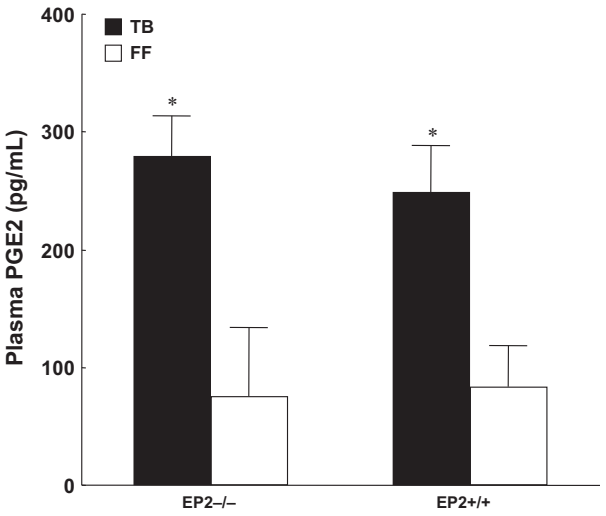

Figure 5. Plasma PGE_2 concentration in tumor-bearing $EP_2^{-/-}$ and $EP_2^{+/+}$ mice compared to sham controls (FF) at the end of experiment (day 10) (mean ± SEM, *$P < 0.01$; seven animals in each group).

Pronounced alteration was observed in $EP_2^{+/+}$ groups due to larger tumors and whole-body water retention. Water retention did not occur in $EP_2^{-/-}$ mice (Fig. 3). Fat-free carcass dry weight was significantly preserved in $EP_2^{-/-}$ tumor-bearing mice compared to wild-type $EP_2^{+/+}$ tumor-bearing mice ($P < 0.001$; Fig. 4), while whole-body fat did not differ between $EP_2^{-/-}$ and wild-type tumor-bearing mice. Plasma PGE_2 levels were similarly elevated

Table 1. mRNA transcripts related to TGF-β signaling with altered levels in hypothalamic tissue from MCG 101 tumor-bearing $EP_2^{-/-}$ versus $EP_2^{+/+}$ mice at the end of experiment (day 10).

Gene name	Gene symbol	NCBI gene ID	Fold change	Regulation
SMAD family member 7	*Smad7*	17131	1.8	Up
Protein Kinase C, delta	*Prkcd*	18753	1.7	Up
Adaptor-related protein complex2, beta 1 subunit	*Ap2b1*	71770	1.6	Up
Mitogen-activated protein kinase kinase 6	*Map2k6*	26399	1.5	Up
Lympoid enhancer-binding protein	*Lef1*	16842	1.6	Down

Table 2. Relative concentrations of Cox 1 and Cox 2 mRNA transcripts in hypothalamic tissue from $EP_2^{-/-}$ and $EP_2^{+/+}$ MCG 101 tumor-bearing mice (TB) and sham-implanted controls (FF) at the end of experiment (day 10, mean ± SEM).

	$EP2^{+/+}$	$EP2^{-/-}$
Cox 1		
TB	1.06 ± 0.11[a]	1.32 ± 0.08[a]
FF	2.24 ± 0.12	1.99 ± 0.07
Cox 2		
TB	3.17 ± 0.85[b]	4.82 ± 0.9[c]
FF	1.73 ± 0.21	1.39 ± 0.19

[a]$P < 0.001$ versus corresponding FF control of same genetic type.
[b]$P < 0.15$ versus corresponding FF control of same genetic type.
[c]$P < 0.01$ versus corresponding FF control of same genetic type.

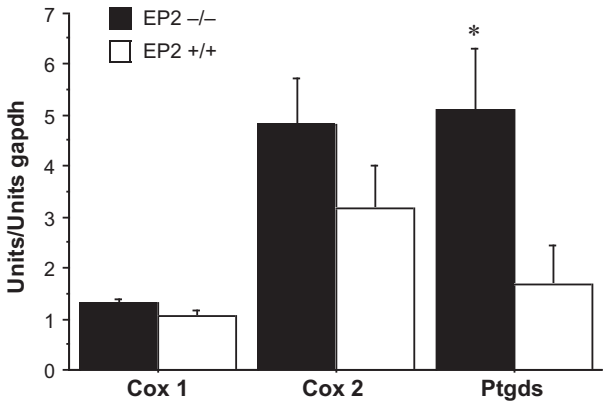

Figure 6. Levels of hypothalamic Cox-1, Cox-2, and Prostaglandin D_2 synthase, mRNA in tumor-bearing $EP_2^{-/-}$ and $EP_2^{+/+}$ mice at the end of experiment (day 10) (mean ± SEM, *$P < 0.05$; seven animals in each group).

in tumor-bearing mice compared to controls in both $EP_2^{-/-}$ and $EP_2^{+/+}$ mice (Fig. 5).

RNA expression in brain hypothalamus

Microarray analysis of pooled extracts of hypothalami from tumor-bearing $EP_2^{-/-}$ mice ($n = 7$) relative to tumor-bearing $EP_2^{+/+}$ animals ($n = 7$) showed differences in mRNA gene expression. We identified 182 genes that had above 1.5-fold change in relative expression between groups; 38 entities had above twofold difference (8 up- and 30 downregulated in $EP2^{-/-}$ vs. $EP2^{+/+}$ mice). The gene list with 1.5-fold changed genes was used for Pathway- and gene ontology analyses to find enriched pathways and categories of genes. Gene ontology search showed a significant match with the GO category GO:0048511 "Rhythmic processes" which contains genes involved in generation and maintenance of rhythms in the physiology of an organism. Additional significant matches were found with Wikipathways "IL2 signaling" $P < 0.05$ (two matching genes) and "TGFβ receptor signaling" $P < 0.001$ (five matching genes, Table 1). We also found *Platg2f*, coding for Phospholipase A_2 (down 2.2) and *Ptgds*; coding for Prostaglandin D_2 synthase (up 2.2), among the genes with large change in expression between groups (TB $EP2^{+/+}$ vs. TB $EP2^{-/-}$).

By real-time PCR we confirmed changes in *Ptgds* (Prostaglandin D_2 synthase) and extended our analysis to include additional genes relevant for prostaglandin production *Ptgs*1 (Cox1), *Ptgs2* (Cox2) ($n = 7$/group). Hypothalamic Cox1 levels were significantly lower while Cox2 levels were increased in tumor-bearing mice compared to controls (Table 2), while Cox2 expression was not significantly altered between tumor-bearing $EP_2^{-/-}$ and $EP_2^{+/+}$ mice (Fig. 6). Prostaglandin D_2 synthase was, however, significantly increased in $EP_2^{-/-}$ tumor-bearing mice compared to $EP_2^{+/+}$ tumor-bearing animals (Fig. 6).

Discussion

In the present study, we examined the role of the EP_2 receptor in anorexia development in mice carrying tumors that induce anorexia/cachexia and released increased levels of PGE_2. We confirmed the results previously observed in the wild-type tumor-bearing groups, where exposure to MCG 101 over 10 days caused reductions of food intake and fat-free carcass weight (Cahlin et al. 2000; Wang et al. 2005a,c). We also found that host

absence of EP_2 receptors retarded MCG 101 tumor growth and maintained food intake and fat-free carcass weight. Genetic knockout of host EP_2 receptors lead to significant changes in expression of mRNA transcripts related to prostanoid production in brain hypothalamus.

In the MCG 101 model the tumor cells produce prostaglandin E_2, which consequently leads to elevated plasma levels of PGE_2 (Lönnroth et al. 1995). Although prostaglandin E_2 is suggested to cross the blood–brain barrier our previous study found no elevation of PGE_2 or its metabolites in cerebrospinal fluid (Ruud et al. 2013). However, indomethacin treatment decreased anorexia concomitant with normalized plasma PGE_2 levels (Wang et al. 2005a), suggesting COX dependency. We have previously suggested that anorexia is dependent on COX-1 expression rather than COX-2 in this model, since a COX-1 inhibitor delayed onset of anorexia while a selective COX-2 inhibitor was without such effect (Ruud et al. 2013). In the present experiments we found no change in relative expression of either COX-1 or COX-2 mRNA in hypothalamus from tumor-bearing EP_2 receptor knockouts compared to tumor-bearing wild-type mice. However, both COX-1 and COX-2 mRNA expressions were significantly altered relative to sham-treated mice. Seen together, it appears that prostaglandins attenuate appetite and stimulate tumor growth which leads to overt cachexia. However, it remains to be determined whether systemic prostaglandins or brain PG production are of relevance. Likely, systemic PGE_2 stimulates tumor growth while hypothalamic PGE_2 production promotes anorexia. In earlier experiments we reported that loss of host EP_1 or EP_3 receptors did not alter anorexia in mice carrying MCG 101 tumors despite effects on tumor growth and body composition by indomethacin treatment (Wang et al. 2005a). Moreover, food intake was improved by short-term treatment by Cox-inhibitors without any effects on tumor size (Ruud et al. 2013). Such findings suggest separate effects of systemic and brain PG production and/or signaling linked to anorexia/cachexia secondary to tumor growth.

To identify other potential CNS mechanisms behind altered anorexia in EP_2 receptor knockout mice we performed microarray analyses of hypothalamic extracts from tumor-bearing $EP_2^{-/-}$ mice relative to $EP_2^{+/+}$ animals. In total, there was a 1.5-fold change difference in expression of around 180 genes. A metabolic pathway search revealed possible involvement of TGFβ signaling, which is associated with inflammatory response and reported to regulate COX/PGE_2 levels, also in CNS (Luo et al. 1998; Minghetti et al. 1998; Matsumura et al. 2009; Fang et al. 2014), although our mice did not display altered COX mRNA levels., However, we found changed expression of other genes directly involved in PG production, such as increased amount of mRNA for Prostaglandin D_2 synthase, and decreased expression of Phospholipase A_2 from hypothalami of tumor-bearing $EP_2^{-/-}$ mice compared with $EP_2^{+/+}$ animals. Thus, reduced expression of Phospholipase A_2 could reflect adaptation of PG production in the brain secondary to lack of EP_2 receptors, contributing to improved food intake, although CNS levels of prostaglandins were not measured in present experiments.

PGD_2 and PGE_2 are positional isomers and have several opposing effects in physiological processes as sleep, body temperature, and feeding behavior (Kandasamy and Hunt 1990; Hayaishi 1991; Ohinata and Yoshikawa 2008). PGE_2 and PGD_2 are produced from the same precursor, PGH_2, and is then converted to PGE_2/PGD_2 by specific enzymes. PGE_2 is produced by the different isoforms of Prostaglandin E_2 synthases whereas Prostaglandin D_2 synthase produces PGD_2. Recent findings report that central administration of PGD_2 was associated with stimulation of food intake (Ohinata et al. 2008). Moreover, intraventricularly administered PGD_2 was reported to stimulate food intake via DP_1 receptor activation (Ohinata et al. 2008). The orexigenic effect of PGD_2 was suggested to stimulate food intake via activation of NPY Y_1 (Ohinata et al. 2008), the most orexigenic of the NPY receptors (Blomqvis and Herzog 1997), and increased mRNA levels of Prostaglandin D_2 synthase were found in brain tissue of fasted mice as well as in food-restricted rats, without similar increases in tumor-bearing animals, supporting its role in appetite control (Ohinata et al. 2008; Pourtau et al. 2011). Therefore, it is plausible that maintained food intake in the $EP_2^{-/-}$ tumor mice was induced by increased DP_1 receptor activity.

Our present and previous results suggest that host EP receptors are involved in control of tumor growth. In the present study, loss of host EP_2 receptors reduced tumor growth which was also observed in our previous studies on EP_1-deficient mice, whereas a lack of EP_3 receptors increased tumor growth (Wang et al. 2005a). Earlier preclinical and clinical studies, suggest a role for cyclooxygenases and prostaglandins in tumor progression, although their downstream signaling is still not well understood. Our finding of reduced MCG 101 tumor growth agree with findings of reduced tumor growth in several other models, such as the syngenic colorectal cancer cell line MC26 as well as Lewis lung carcinoma in hosts lacking EP_2 receptors (Yang et al. 2003). The importance of EP_2 receptors for cancer cell proliferation has also been demonstrated using newly discovered selective EP_2 antagonists (Jiang and Dingledine 2013b).

In conclusion, we demonstrate the importance of EP_2 receptors for anorexia, cachexia progression in tumor-

bearing mice, possibly mediated by altered balance of PGE_2/PGD_2 production in brain hypothalamus. Our results of reduced MCG 101 tumor growth are consistent with previous studies showing the importance of EP_2 receptor signaling in tumor proliferation.

Acknowledgments

We are grateful to Professors Kent Lundholm and Anders Blomqvist for their support of this study.

Conflict of Interest

The authors declare that they have no competing interests.

References

Blomqvis, A. G., and H. Herzog. 1997. Y-receptor subtypes–how many more? Trends Neurosci. 20:294–298.

Cahlin, C., A. Korner, H. Axelsson, W. Wang, K. Lundholm, and E. Svanberg. 2000. 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 Res. 60:5488–5493.

Cahlin, C., C. Lonnroth, A. Arvidsson, S. Nordgren, and K. Lundholm. 2008. Growth associated proteins in tumor cells and stroma related to disease progression of colon cancer accounting for tumor tissue PGE2 content. Int. J. Oncol. 32:909–918.

Eden, E., L. Lindmark, I. Karlberg, and K. Lundholm. 1983. Role of whole-body lipids and nitrogen as limiting factors for survival in tumor-bearing mice with anorexia and cachexia. Cancer Res. 43:3707–3711.

Fang, L., H. M. Chang, J. C. Cheng, P. C. Leung, and Y. P. Sun. 2014. TGF-beta1 induces COX-2 expression and PGE2 production in human granulosa cells through Smad signaling pathways. J. Clin. Endocrinol. Metab. 99:E1217–E1226.

Furuyashiki, T., and S. Narumiya. 2011. Stress responses: the contribution of prostaglandin E(2) and its receptors. Nat. Rev. Endocrinol. 7:163–175.

Hayaishi, O. 1991. Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D2 and E2. FASEB J. 5:2575–2581.

Jiang, J., and R. Dingledine. 2013a. Prostaglandin receptor EP2 in the crosshairs of anti-inflammation, anti-cancer, and neuroprotection. Trends Pharmacol. Sci. 34:413–423.

Jiang, J., and R. Dingledine. 2013b. Role of prostaglandin receptor EP2 in the regulations of cancer cell proliferation, invasion, and inflammation. J. Pharmacol. Exp. Ther. 344:360–367.

Kandasamy, S. B., and W. A. Hunt. 1990. Involvement of prostaglandins and histamine in radiation-induced temperature responses in rats. Radiat. Res. 121:84–90.

Kiriyama, M., F. Ushikubi, T. Kobayashi, M. Hirata, Y. Sugimoto, and S. Narumiya. 1997. Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 122:217–224.

Laflamme, N., S. Lacroix, and S. Rivest. 1999. An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. J. Neurosci. 19:10923–10930.

Lönnroth, C., G. Svaninger, J. Gelin, C. Cahlin, B.-M. Iresjö, E. Cvetkovska, et al. 1995. 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.

Lundholm, K., S. Edstrom, L. Ekman, I. Karlberg, A. C. Bylund, and T. Schersten. 1978. A comparative study of the influence of malignant tumor on host metabolism in mice and man: evaluation of an experimental model. Cancer 42:453–461.

Luo, J., J. A. Lang, and M. W. Miller. 1998. Transforming growth factor beta1 regulates the expression of cyclooxygenase in cultured cortical astrocytes and neurons. J. Neurochem. 71:526–534.

Matsumura, T., T. Suzuki, K. Aizawa, D. Sawaki, Y. Munemasa, J. Ishida, et al. 2009. Regulation of transforming growth factor-beta-dependent cyclooxygenase-2 expression in fibroblasts. J. Biol. Chem. 284:35861–35871.

Minghetti, L., E. Polazzi, A. Nicolini, and G. Levi. 1998. Opposite regulation of prostaglandin E2 synthesis by transforming growth factor-beta1 and interleukin 10 in activated microglial cultures. J. Neuroimmunol. 82: 31–39.

Ohinata, K., and M. Yoshikawa. 2008. Central prostaglandins in food intake regulation. Nutrition 24:798–801.

Ohinata, K., K. Suetsugu, Y. Fujiwara, and M. Yoshikawa. 2006. Activation of prostaglandin E receptor EP4 subtype suppresses food intake in mice. Prostaglandins Other Lipid Mediat. 81:31–36.

Ohinata, K., K. Takagi, K. Biyajima, Y. Fujiwara, S. Fukumoto, N. Eguchi, et al. 2008. Central prostaglandin D(2) stimulates food intake via the neuropeptide Y system in mice. FEBS Lett. 582:679–684.

Patterson, T. A., E. K. Lobenhofer, S. B. Fulmer- Smentek, P. J. Collins, T. M. Chu, W. Bao, et al. 2006. Performance comparison of one-color and two-color platforms within the MicroArray Quality Control (MAQC) project. Nat. Biotechnol. 24:1140–1150.

Plata-Salaman, C. R. 1999. 1998 Curt P. Richter Award. Brain mechanisms in cytokine-induced anorexia. Psychoneuroendocrinology 24:25–41.

Pourtau, L., S. Leemburg, P. Roux, T. Leste-Lasserre, P. Costaglioli, B. Garbay, et al. 2011. Hormonal, hypothalamic and striatal responses to reduced body weight gain are attenuated in anorectic rats bearing small tumors. Brain Behav. Immun. 25:777–786.

Ruud, J., A. Nilsson, L. Engstrom Ruud, W. Wang, C. Nilsberth, B. M. Iresj, et al. 2013. Cancer-induced anorexia in tumor-bearing mice is dependent on cyclooxygenase-1. Brain Behav. Immun. 29:124–135.

Tilley, S. L., L. P. Audoly, E. H. Hicks, H. S. Kim, P. J. Flannery, T. M. Coffman, et al. 1999. Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E2 receptor. J. Clin. Invest. 103:1539–1545.

Wang, W., C. Lonnroth, E. Svanberg, and K. Lundholm. 2001. Cytokine and cyclooxygenase-2 protein in brain areas of tumor-bearing mice with prostanoid-related anorexia. Cancer Res. 61:4707–4715.

Wang, W., M. Andersson, C. Lonnroth, E. Svanberg, K. Lundholm. 2005a. Anorexia and cachexia in prostaglandin EP1 and EP3 subtype receptor knockout mice bearing a tumor with high intrinsic PGE2 production and prostaglandin related cachexia. J. Exp. Clin. Cancer Res. 24:99–107.

Wang, W., M. Andersson, C. Lonnroth, E. Svanberg, and K. Lundholm. 2005b. Prostaglandin E and prostacyclin receptor expression in tumor and host tissues from MCG 101-bearing mice: a model with prostanoid-related cachexia. Int. J. Cancer 115:582–590.

Wang, W., E. Svanberg, D. Delbro, and K. Lundholm. 2005c. NOS isoenzyme content in brain nuclei as related to food intake in experimental cancer cachexia. Brain Res. Mol. Brain Res. 134:205–214.

Yang, V. W., J. M. Shields, S. R. Hamilton, E. W. Spannhake, W. C. Hubbard, L. M. Hylind, et al. 1998. Size-dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis. Cancer Res. 58:1750–1753.

Yang, L., N. Yamagata, R. Yadav, S. Brandon, R. L. Courtney, J. D. Morrow, et al. 2003. Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP2 receptor. J. Clin. Invest. 111:727–735.

Zhang, J., and S. Rivest. 1999. Distribution, regulation and colocalization of the genes encoding the EP2- and EP4-PGE2 receptors in the rat brain and neuronal responses to systemic inflammation. Eur. J. Neurosci. 11:2651–2668.