Human cytomegalovirus infection is correlated with enhanced cyclooxygenase-2 and 5-lipoxygenase protein expression in breast cancer

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Abstract
Purpose While enhanced expression of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) and their derived metabolites is associated with breast cancer (BC) risk, the precise link between BC carcinogenesis and enhanced inflammatory activity remains to be clarified. Human Cytomegalovirus (HCMV) may induce expression of COX-2 and 5-LO and is frequently found in breast cancer biopsies. Thus, we investigated whether there is an association between HCMV proteins and expression of COX-2 and 5-LO in human BC tissue and BC cell lines.

Materials and methods Paraffin embedded biopsies obtained from 49 patients with breast cancer and 26 tissue samples from adjacent, benign breast tissues were retrospectively examined for HCMV-immediate early (IE), HCMV-Late (LA), COX-2, and 5-LO proteins by immunohistochemistry. In vitro, uninfected and HCMV-infected BC cell lines were examined for COX-2 and 5-LO transcripts and proteins by PCR and flow cytometry.

Results Extensive expression of COX-2, 5-LO and HCMV-IE proteins were preferentially detected in BC samples. We found a statistically significant concordant correlation between extensive HCMV-IE and COX-2 (P < 0.0001) as well as with HCMV-IE and 5-LO (P=0.0003) in infiltrating BC. In vitro, HCMV infection induced COX-2 and 5-LO transcripts and COX-2 proteins in MCF-7 cells (P=0.008, P=0.018, respectively). In MDA-MB-231 cells that already had high base line levels of COX-2 expression, HCMV induced both COX-2 and 5-LO proteins but not transcripts.

Conclusion Our findings demonstrate a significant correlation between extensive HCMV-IE protein expression and overexpression of COX-2 and 5-LO in human breast cancer.

Keywords Human cytomegalovirus · Breast cancer · 5-LO · COX-2 · MCF-7 · MDA-MB-231
Introduction

Breast cancer (BC) is the most common cancer in women worldwide and although implementation of novel therapies and early detection has improved quality of life and decreased mortality rates for some BC subtypes, the overall incidence of BC continues to increase (Jemal et al. 2011). Of high concern, the overall survival has not improved significantly albeit new therapies for patients with metastatic BC have been introduced during the past decades (https://www.kreftregisteret.no/globalassets/publikasjoner-og-rapporter/cancer-in-norway/cancer_in_norway_2009.pdf), suggesting that alternative treatment options are urgently needed. Somatic mutations in breast cancer-associated genes are today considered to be one of the main causes and drivers of BC. In addition, epigenetic and genetic alterations in key breast cancer genes such as BRCA-1/2 and their downstream regulation of DNA repair mechanisms, or in genes with impact on epithelial cell proliferation, differentiation and migration are suggested to promote breast cancer carcinogenesis (Petruccelli et al. 1993). Emerging evidence also highlights a role of the tumor microenvironment to induce pre-malignancies and promote tumor progression. Stroma cells like specialized mesenchymal cells and myo-fibroblasts, endothelial cells and infiltrating leukocytes with capacity to produce inflammatory factors may promote breast cancer carcinogenesis and affect tumor progression and metastasis formation (Balkwill et al. 2005; Coussens and Werb 2002; de Visser et al. 2006). Inflammatory cells present in the tumor microenvironment support tumor growth and enhanced malignancy, by releasing cytokines, chemokines and growth factors. Their increased production of reactive oxygen species also further enhance inflammation, oxidative DNA damage and impair DNA repair mechanisms (Coussens and Werb 2002). Therefore, chronic inflammation is considered a risk factor for breast cancer development, and added inflammation to their revised version of the Hallmarks of Cancer (Hanahan and Weinberg 2011).

Eicosanoids are potent inflammatory mediators that are produced from arachidonic acid by cyclooxygenases (COXs) and lipooxygenase (LO). Enhanced inflammation via COX-2 and 5-LO promotes tumorigenesis (Chen and Smyth 2011) and is associated with poor prognosis of several cancer forms (Dreyling et al. 1986; Hennig et al. 2002; Steele et al. 1999). Over-expression of COX-2 was observed in 40% of patients with invasive breast carcinoma and correlated with poor prognosis (Denkert et al. 2003; Howe 2007). Selective COX-2 inhibitors reduce the risk of breast cancer, suppress breast cancer cell migration and invasion, and exhibit strong anti-neoplastic effects in animal models (Wang and DuBois 2010; Harris et al. 2006, 2014). COX-2 inhibitors are, therefore, currently evaluated as potential new cancer therapies, especially in epithelial-derived malignancies. A randomised phase II study of 111 postmenopausal women with advanced breast cancer indicated a trend towards a longer duration of clinical benefit in the combination arm (median, 96.6 weeks vs 49 weeks) compared to the exemestane monotherapy arm (Dirix et al. 2008). Inhibition of 5-LO activity in several breast cancer cell lines also resulted in growth inhibition and enhanced apoptosis, but 5-LO inhibitors have not yet been evaluated in clinical trials in breast cancer patients (Avis et al. 2001).

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that is proposed to be highly prevalent in different cancer forms including breast (Harkins et al. 2010; Taher et al. 2013, 2014), colon (Dimberg et al. 2013; Harkins et al. 2002; Tafvizi and Fard 2014) and prostate cancer (Samanta et al. 2003), glioblastoma multiforme (Cobbs et al. 2002; Lucas et al. 2011; Rahbar et al. 2015), medulloblastoma (Baryawno et al. 2011) and neuroblastoma (Wolmer-Solberg et al. 2013). HCMV is also present in sentinel lymph node metastases of breast cancer and in brain metastases originating from breast and colon cancer, while healthy tissues surrounding HCMV positive primary tumors are generally HCMV negative. In breast cancer, HCMV was detected in 92% of primary tumors, in 94% of sentinel lymph nodes, and in 99% of brain metastases originating from breast cancer (Taher et al. 2013, 2014). These observations suggest a concerning association between HCMV and malignant cell growth. However, it remains to be clarified whether these findings represent an epiphenomenon or indicate that HCMV infections induce tumor-promoting mechanisms of relevance for local tumor progression.

A close link between HCMV infections and inflammation has been established in the literature. Thus, latent HCMV can be reactivated by inflammation and the virus further promotes inflammation by inducing expression of both COX-2 and 5-LO (Benard et al. 2014; Qiu et al. 2008; Hooks et al. 2006). Results from a mouse model based on virus-induced adenocarcinoma (Bongers et al. 2010), confirmed production of inflammatory factors such as IL-10, TGF-β, IL-1β, IL-8, IL-6, MIP-1α, MIP-1β, and RANTES (Bodaghi et al. 1998; Kotenko et al. 2000; Reeves et al. 2005; Söderberg-Nauclér et al. 1997) to be involved. We earlier showed that COX-2 is almost exclusively expressed by HCMV-infected cells in medulloblastoma and that COX-2 inhibition acts to inhibit HCMV, which led to decreased tumor growth in an animal model (Baryawno et al. 2011; Schroer and Shenk 2008). In addition to inducing inflammation, HCMV may directly modulate tumor cells by affecting intracellular pathways involved in cell cycle regulation, epigenetic regulation of gene expression, cellular invasion, angiogenesis, immune evasion, and apoptosis (Cinatl et al. 2004a, b; Slinger 2010; Vossen et al. 2002), all highly relevant in tumor biology.
The main objective of this study was to investigate a potential association between HCMV infection and simultaneous expression of COX-2 and 5-LO in BC. We examined the expression of HCMV proteins, COX-2 and 5-LO, in tissue samples obtained from BC and in adjacent normal breast tissues and investigated whether the activity level of HCMV was associated with inflammatory markers and impaired clinical outcome. In additional in vitro experiments, we assessed whether HCMV could affect the expression of 5-LO and COX-2 in well-established BC cell lines.

Materials and methods

Study design

Paraffin-embedded tissue specimens of infiltrating BC (n = 75) and adjacent normal breast tissue (n = 26) were retrospectively obtained from 49 patients who underwent surgery at Akershus University Hospital, Oslo, Norway during 2011. Clinical data (Table 1) were provided by the Departments of Oncology and Pathology at Akershus University Hospital (AHUS). All diagnoses were re-confirmed by an experienced BC pathologist (T.S.) at AHUS. The median age at surgery was 58.7 years. Most patients underwent mastectomy (61%), while 35% had breast-conserving surgery, and 4% bilateral surgery. All patients received standard adjuvant treatment according to the Norwegian guidelines approved at the time of surgery (Table 1); (http://www.nbcg.no).

Immunohistochemistry

Tissue microarrays were created, and all tissues were sectioned (4 µm) and analyzed by immunohistochemical techniques optimized in our laboratory. Detection of HCMV proteins was done as described previously with only minor modifications (Taher et al. 2013). Tissue specimens were deparaffinized in xylene (Sigma Aldrich), rehydrated in an ethanol (Apoteket Farmaci), and washed in Tris-buffered saline (TBS) containing Triton X-100 (Substrate Department, Karolinska University Hospital). Antigen retrieval and unmasking was done by heating the tissues in DIVA decloaker buffer (Histolab), pH 6.2, in a pressure cooker (BioCARE) for 15 min. Endogenous peroxidase activity was blocked with peroxidase 1 (Histolab) for 5 min, and nonspecific binding was blocked with Sniper (Histolab) for 16 min at room temperature. The tissue sections were then incubated with antibodies against HCMV-IE and HCMV-LA (IgG2a, Merck), COX-2 (CellSignaling), 5-LO (Abcam), and cytokeratins 5, 6, 8, 17, and 19 (IgG1, Dako). An antibody against cytookeratin 20 (IgG2a, Chemicon International) and rabbit IgG (Biocare Medical) served as negative controls. Paraffin-embedded tissue

| Table 1 Patients characteristic | n (%) |
|---------------------------------|-------|
| **Patient characteristics**     |       |
| Patients                        | 49    |
| Median age (years)              | 49    |
| Median age at surgery (years)   | 58.7  |
| **Type of surgery**             |       |
| Breast conserving               | 17 (35)|
| Ablation                        | 30 (61)|
| Bilateral                       | 2 (4) |
| **Histology**                   |       |
| Infiltrating ductal cancer      | 41 (84)|
| Infiltrating lobular cancer     | 4 (8)  |
| Medullar cancer                 | 0 (0)  |
| Multiple cancer types           | 1 (2)  |
| Mucinous subtype                | 3 (6)  |
| **Grades (n = 48)**             |       |
| I                               | 6 (13) |
| II                              | 17 (35)|
| III                             | 25 (52)|
| **T stadium (n = 49)**          |       |
| T1                              | 25 (51) |
| T2                              | 24 (49) |
| **N stadium (n = 49)**          |       |
| N0                              | 29 (59.2) |
| N1                              | 11 (22.4) |
| N2                              | 7 (14.3) |
| N3                              | 2 (4.1) |
| **ER expression**               |       |
| Positive (≥ 1%)                 | 35 (71) |
| Negative                        | 14 (29) |
| **PGR expression**              |       |
| Positive (≥ 10%)                | 23 (47) |
| Negative                        | 26 (53) |
| **HER2 expression**             |       |
| Positive (IHC3 + or FISH +)     | 8 (16) |
| Negative                        | 41 (84) |
| **Ki-67 labeling index (n = 42)** |       |
| < 1%                            | 1 (2.4) |
| 1–30%                           | 22 (52.4) |
| > 31–100%                       | 19 (45.2) |
| **Menopause (n = 18)**          |       |
| Premenopause                    | 5 (28) |
| Postmenopause                   | 13 (72) |
| **Neoadjuvant treatment**       |       |
| Yes                             | 1 (2) |
| No                              | 48 (98) |
| **Adjuvant chemotherapy**       |       |
| No chemotherapy                 | 19 (39) |
| FEC60×6                         | 2 (4) |
| FEC60×4 followed by taxanes (every 3 weeks) | 19 (39) |
| FEC100×4 followed by taxanes (every 3 weeks) | 9 (18) |
section from HCMV-infected placenta was used as positive control and from HCMV negative breast cancer patient as negative control for IHC.

During the staining procedure a few of the tissue sections were lost.

HCMV, COX-2, and 5-LO staining was evaluated as described previously (Taher et al. 2013), and according to the estimated percentage of cells expressing HCMV or COX-2 or 5-LO proteins: negative (0%), grade 1 (< 25%), grade 2 (≥ 25–50%), grade 3 (≥ 50–75%), and grade 4 (≥ 75%). To ensure a sufficient number of cases in each category for statistical analysis, tumors were considered as HCMV-negative or as having focal HCMV infection (< 50% positive cells) or extensive infection (≥ 50% positive cells). Immunohistochemical (IHC) staining for HCMV was evaluated and graded by a senior scientist (A.R.) without access to the clinical records at Karolinska Institutet, Stockholm, while IHC staining for Ki-67 was performed and evaluated at the Department of Pathology, Akershus University Hospital.

### RNA extraction and quantitative real-time PCR (qPCR)

MCF-7 and MDA-MB-231 cells were infected with HCMV VR1814 at multiplicity of infection (MOI) of 5 and were collected at different times post-infection. RNA was extracted from lysed cells using RNeasy Mini Kit (Qiagen) according to manufacturer’s instructions and cDNA was synthesized using random primers and the high-capacity cDNA reverse transcription kit (Applied Biosystems). Gene expression levels were quantified by real-time PCR using TaqMan Fast Universal PCR Master Mix (Life Technologies) and the following specific TaqMan probes: COX-2 (PTGS2, assay ID Hs00153133_m1), 5-LO (ALOX5, assay ID Hs00167536_m1), HCMV IE, and human β2-microglobulin (B2M, assay ID, Hs00984230_m1) (Life Technologies). The PCR was performed using a 7900HT Fast Real-Time PCR system (Applied Biosystems). The endogenous control B2M was used for normalization and relative expression was determined by the $2^{-ΔΔCt}$ method. (Three separate experiments were performed).

### Flow cytometric analysis (FACS)

MCF-7 and MDA-MB-231 cells non-infected or infected with HCMV MOI of 5 were fixed at 6 days post infection (dpi). Cells were treated in Fix &Perm Medium A and B according to the supplier’s instruction (Molecular Probes) and co-stained with primary antibodies HCMV IE (Merck) and COX-2 (CellSignaling) or 5-LO (Abcam) diluted in phosphate-buffered saline (PBS) containing 1% BSA (Sigma) for 45 min at 4 °C. After washing with PBS, cells were incubated with secondary Alexa Fluor 633 goat anti-mouse antibodies (Life Technologies) and Alexa Fluor 488 anti-rabbit antibodies (Invitrogen). Rabbit IgG (Biocare Medical) and mouse IgG2a (Dako) served as negative controls.
at different levels in breast cancer (BC) tissue specimens from all 49 patients and HCMV-LA protein was found in 22% (11/49) of BC patients. HCMV IE protein expression was extensive (defined as > 50% positive cells) in 73% (55/75) of BC tissue specimens, but only in 8% (2/26) of adjacent breast tissue specimens. This cohort of breast cancer patients was previously investigated for HCMV protein expression and association with estrogen receptor-alpha (ER) and progesterone receptor (PGR) expression and the results were reported earlier (Rahbar et al. 2017). Most of tumor specimens exhibited low expression of HCMV LA proteins; extensive HCMV-LA expression was only present in 3 of 75 (4%) of BC specimens, and in none of adjacent breast tissue specimens.

COX-2 protein expression was extensive in 58% (48/67) of BC specimens and in 8% (2/26) of adjacent non-tumor breast specimens. 5-LO protein expression was extensive in 53% (40/75) of BC specimens and in 31% (9/29) of adjacent non-tumor breast specimens. Expression of HCMV-IE protein was detected in infiltrated BC (a). Extensive HCMV-IE and COX-2 were detected in majority of infiltrated BC (b, c).

Statistical analysis

All analyses were done with GraphPad Prism version 6; \( P < 0.05 \) was considered statistically significant. Nonparametric Pearson correlation assay was used to assess statistical significance of correlation between different grades of HCMV-IE, COX-2, and 5-LO in BC tissues. Two-tailed Student \( t \) test was used for analysis of in vitro data, presented as mean standard deviation.

Results

High prevalence of HCMV-IE, COX-2, and 5-LO proteins in BC, but not in adjacent non-malignant breast tissues

Expression of HCMV-IE, HCMV-LA, COX-2, and 5-LO proteins was analyzed in available tissue sections by immunohistochemistry. HCMV IE protein was detected controls. Acquisition was performed using Flow cytometry (FACS) and data were analyzed by Summit 4.3 software. (Three separate experiments were performed).

Fig. 1 Detection of HCMV-IE and HCMV-LA in infiltrated BC and adjacent normal breast tissue by immunohistochemical staining (IHC). Extensive HCMV-IE but not HCMV-LA was frequently detected in infiltrated BC (a). Extensive HCMV-IE and COX-2 were detected in majority of infiltrated BC (b, c).
Significant positive correlation between HCMV-IE protein, COX-2 and 5-LO expression

We further assessed whether there was a correlation between HCMV protein expression, COX-2, and 5-LO expression. A positive correlation was found between extensive HCMV-IE protein expression and extensive COX-2 or 5-LO protein expression in breast cancer tissue specimens (49% and 48%, respectively) (Fig. 2a–d). Significantly higher number of BC tissues had equal levels of both HCMV-IE/COX-2 and HCMV-IE/5-LO protein expression (concordant); 76% (51/67, \( P < 0.0001 \)) and 69% (52/75, \( P = 0.0003 \)), respectively (Fig. 2a–d). In adjacent non-tumor breast tissue, we instead found concordant levels of focal HCMV-IE/COX-2 and HCMV-IE/5-LO in 92% (24/26), and 83% (24/29) of the samples, respectively (Fig. 3a–d). Extensive expression of HCMV-IE and COX-2/5-LO was located in the same areas within the tissue sections. But in case of focal expression,
these proteins were mainly expressed not only in the inflammatory cells but also in some tumor cells and in the cells of vessel walls.

Clinical data including Ki-67 labeling index, nodal status, and tumor stadium for BC patients are shown in Table 1. We found no association between HCMV-IE, COX-2 and 5-LO and lymph node involvement, tumor size or Ki-67 index (Supplemental Figs. 2, 3). At the time of our investigation, 92% (45/49) of BC patients were alive and median overall survival was 4.4 years. We also found no association between extensive HCMV infection and these inflammatory markers with patient outcome, but as only few patients in this cohort had relapsed or died, these results are still premature and somewhat uncertain.

**HCMV induces COX-2 and 5-LO transcript levels in MCF-7 but not in MDA-MB-231 breast cancer cell lines**

To assess whether HCMV can induce COX-2 and 5-LO expression in breast cancer cell lines, we infected MCF-7 and MDA-MB-231 with HCMV strain VR1814 at MOI of 5 and analyzed transcript levels of COX-2 and 5-LO by
qPCR at 1, 3, and 6 dpi. In MCF-7 cells, HCMV infection resulted in a significantly induced COX-2 transcript expression at 3 and 6 dpi ($P=0.015$ and $P=0.008$, respectively), and 5-LO at 6 dpi ($P=0.018$) (Fig. 4a, b). However, HCMV infection had no effect on COX-2 or 5-LO transcript levels in MDA-MB-231 cells (Fig. 4c, d) as assessed with qPCR. Uninfected and HCMV-infected BC cell lines MCF-7 and MDA-MB-231 at 6 dpi were further analyzed for protein expression of COX-2 and 5-LO by FACS. Baseline COX-2 protein levels were higher in MDA-MB-231 cells compared with MCF-7 cells (Figs. 5, 6). COX-2 was expressed in 58% and 40% of MDA-MB-231 and MCF-7 cells, respectively (Figs. 5, 6). 5-LO was expressed in only 6% of MDA-MB-231 and MCF-7 cells (Figs. 5, 6).

The number of cells expressing COX-2 increased from 58 to 70% in HCMV-infected MDA-MB-231 and from 40 to 71% in MCF-7 cell population. The number of HCMV-induced 5-LO expressing cells increased from 6 to 29% in MDA-MB-231 population, but did not affect 5-LO expression in MCF-7 cells (Figs. 5, 6). Seventy percent of HCMV infected MDA-MB-231 and MCF-7 cells expressed COX-2 (Figs. 5, 6). Twenty-nine percent of HCMV-infected MDA-MB-231 cells, but only 3% of MCF-7 cells expressed 5-LO (Figs. 5, 6).

**Discussion**

The presence of HCMV, as well as other herpesviruses in different types of cancers has been studied by several groups with variable results (Cianchi et al. 2006; Rahbar et al. 2017). Several studies have reported a very high prevalence of HCMV in breast cancer and linked the virus activity levels to overall survival (Taher et al. 2013; Tsai et al. 2007). Consistent with these observations, we found HCMV protein expression in all breast cancer tissue specimens examined. Moreover, HCMV IE protein expression was extensive (defined as > 50% positive cells) in 72% of breast cancer samples, but only in 8% of adjacent non tumor tissues. These observations give support for a potential role of HCMV in breast cancer progression (Kumar et al. 2018; Rahbar et al. 2017). As it seems, HCMV infections can provide direct effects on tumor cells by expression of viral proteins that affect tumor biology-relevant mechanisms and by virus-induced inflammation. In fact, HCMV seems to contribute to all established Hallmarks of Cancer (Soroceanu and Cobbs 2011).

In support of this statement, we detected extensive expression of the inflammatory factors COX-2 and 5-LO in infiltrating breast cancer (58%, 53%, respectively), but less pronounced activity of these inflammatory mediators in adjacent non-tumor breast tissues (8% and 31%, respectively). These inflammatory factors were expressed in the tumor and in inflammatory cells. We also found a concordant extensive expression of COX-2, 5-LO, and HCMV-IE in breast cancer, suggesting that HCMV activity on tumor cells may be linked to COX-2 and 5-LO expression. This hypothesis was further supported by in vitro experiments demonstrating virus-induced transcript of both COX-2 and 5-LO and COX-2 protein expression in HCMV-infected MDA-231 and MCF-7 cells. However, we did not observe higher expression of COX-2 or 5-LO transcripts in HCMV-infected MDA-231 cells, which may be explained by the already high expression levels of COX-2 proteins in the more aggressive cell line MDA-231 that was originally established from a triple negative breast cancer (TNBC). Previous studies have reported high expression levels of COX-2 in TNBCs (Chikman et al. 2014; Mosalpuria et al. 2014), and we recently reported that HCMV protein expression was high in TNBC (Rahbar et al. 2017). Our results confirm previous studies demonstrating increased levels of COX-2, 5-LO, and their metabolites prostaglandins and leukotrienes in HCMV-infected cells both in vitro and in vivo (Maussang et al. 2009; Qu et al. 2008; Hooks et al. 2006; Bongers et al. 2010).

HCMV-induced COX-2 expression is mediated by the constitutively active viral chemokine receptor homologue US28, which promotes inflammation, angiogenesis, and tumor formation (Maussang et al. 2009). US28 promotes angiogenesis and tumor formation via COX-2 induced production of IL-6, phosphorylation of STAT-3, and activation of nuclear factor-kappaB (Maussang et al. 2009). High COX-2 and 5-LO expression and production of inflammatory mediators will also attract inflammatory cells capable of releasing inflammatory factors and reactive oxygen species causing inflammation, oxidative DNA damage, and impaired DNA repair mechanisms (Coussens and Werb 2002). In a mouse model with targeted expression of US28 in colon, mice developed inflammation associated adenocarcinoma (Bongers et al. 2010). Consistent with an important role for the virus to induce COX-2 expression, COX-2 inhibition obstructs HCMV replication (Zhu et al. 2002) and reduces growth of HCMV positive tumors in animal models (Baryawno et al. 2011; Maussang et al. 2009). In addition, combined antiviral therapy targeting HCMV (Valganciclovir) and COX-2 (Celecoxib) prevents HCMV replication and PGE2 production, further reduced medulloblastoma and neuroblastoma cell proliferation in vitro (Baryawno et al. 2011; Wolmer-Solberg et al. 2013) and tumor size in medulloblastoma xenografts (Baryawno et al. 2011).
Fig. 5  The number of cells expressing COX-2 but not 5-LO increased in HCMV infected MCF-7 cell populations compared with uninfected cells analyzed by using FACS. Eighty-two percent of HCMV infected MCF-7 cells express COX-2 protein and only 8.6% of the infected cells expressed 5-LO
cells and the tumor microenvironment may enhance tumor malignancy grade and promote tumor progression.

Epidemiological studies in large patient cohorts have reported that the cancer risk and metastasis development...
is reduced in patients who are treated with non-selective COX inhibitors (Anderson et al. 2002; Burn et al. 2011; Xu 2002). The critical role of inflammatory COX-2 and 5-LO and their biological products, prostaglandin and leukotrienes in inflammation and their ability to stimulate signalling pathways contributing to angiogenesis, tumor growth, and invasiveness, further highlights them as potential targets for cancer therapy. Notably, inhibitors of 5-LO were efficiently used to block proliferation of breast cancer cells and a lipoxigenase inhibitor administrated to one breast cancer patient successfully reverted multiple brain metastasis (Flavin 2007; Hammamieh et al. 2007; Poeckel et al. 2006). In vitro experiments and animal models using specific COX-2 inhibitors show a reduction in proliferation and invasion of breast cancer cells and an effect on tumor development and growth (Bocca et al. 2011; Na et al. 2013; Silva et al. 2012). Consistent with these findings, specific or non-specific inhibition of COX-2 has successfully been used for prevention and therapy of breast cancer (Arun and Goss 2004). Selective COX-2 inhibitors appear to be more protective but in both cases, with a significant reduction in the breast cancer risk (Ashok et al. 2011). However, the inhibition of both COX-2 and 5-LO would have additive benefits by simultaneously targeting both pathways of arachidonic acid metabolism. Indeed, combined treatment with COX-2 and 5-LO inhibitors showed a stronger effect on tumor cell proliferation and induction of apoptosis in colon cancer cells (Che et al. 2016; Cianchi et al. 2006). However, these compounds may also involve a so far not considered anti-viral effect against HCMV to affect tumor growth (Schroer and Shenk 2008; Zhu et al. 2002). As selective COX-2 inhibitors may confer increased risk of cardiovascular diseases and stroke (Trelle et al. 2011; Back et al. 2012; Martinez-Gonzalez and Badimon 2007; Sibbald 2004), there is a need to develop a non-toxic drug with potential to inhibit COX-2 and 5-LO, with therapeutic action in human cancers (Gautam et al. 2017).

In conclusion, we detected higher grades of HCMV-IE, COX-2 and 5-LO in the majority of BC samples than in adjacent non-malignant tissue specimens and a significant correlation between extensive HCMV-IE, COX-2 and 5-LO protein levels in infiltrating BCs. These results suggest that inflammation driven by COX-2 and 5-LO in human BC might be induced by HCMV in some patients and promote tumor progression. Thus, we suggest that anti-viral therapy targeting HCMV and inhibitors targeting COX-2 and 5-LO should be evaluated as new therapeutic options in selected breast cancer patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest and that the results presented in this paper have not been published previously. This cohort of breast cancer patients was previously investigated for HCMV protein expression and association with estrogen receptor-alpha (ER) and progesterone receptor (PRG) expression and the results were reported earlier (Rahbar et al. 2017).

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients gave their written informed consent prior to inclusion. The study protocol was approved by the regional ethical committee of south-east Norway, Oslo, Norway (2008/628-31/2, 577-06-04148, 06118 and 2014/895) and by the ethical committee at the Karolinska Institutet (Dnr: 2008/628-31/2), Stockholm, Sweden. Placental tissues were obtained after informed parental consent and according to the procedures approved by the Regulations of the French Ministry of Health.

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References

Anderson WF, Umar A, Viner JL, Hawk ET (2002) The role of cyclooxygenase inhibitors in cancer prevention. Curr Pharm Des 8:1035–1062
Arun B, Goss P (2004) The role of COX-2 inhibition in breast cancer treatment and prevention. Semin Oncol 31:22–29
Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD (2011) Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast 20:66–70. https://doi.org/10.1016/j.breast.2010.07.004
Avis I et al (2001) Five-lipoxygenase inhibitors can mediate apoptosis in human breast cancer cell lines through complex eicosanoid interactions. FASEB J. https://doi.org/10.1096/fj.00-0866fj e
Back M, Yin L, Ingelsson E (2012) Cyclooxygenase-2 inhibitors and cardiovascular risk in a nation-wide cohort study after the withdrawal of rofecoxib. Eur Heart J 33:1928–1933. https://doi.org/10.1093/eurheartj/ehr421
Balkwill F, Charles KA, Mantovani A (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7:211–217. https://doi.org/10.1016/j.cccr.2005.02.013
Baryawno N et al (2011) Detection of human cytomegalovirus in medulloblastomas reveals a potential therapeutic target. J Clin Invest 121:4043–4055. https://doi.org/10.1172/JCI57147

Benard M et al (2014) Human cytomegalovirus infection induces leukotriene B4 and 5-lipoxygenase expression in human placenta and umbilical vein endothelial cells. Placenta 35:345–350. https://doi.org/10.1016/j.placenta.2014.03.022

Bocca C, Bozzo F, Bassignana A, Miglietta A (2011) Antiproliferative effects of COX-2 inhibitor celecoxib on human breast cancer cell lines. Mol Cell Biochem 350:59–70. https://doi.org/10.1007/s11010-010-0682-4

Bodaghi B et al (1998) Chemokine sequestration by viral chemoreceptors as a novel viral escape strategy: withdrawal of chemokines from the environment of cytomegalovirus-infected cells. J Exp Med 188:855–866

Bongers G et al (2010) The cytomegalovirus-encoded chemokine receptor US28 promotes intestinal neoplasia in transgenic mice. J Clin Invest 120:3969–3978. https://doi.org/10.1172/jci42563

Burn J et al (2011) Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet 378:2081–2087. https://doi.org/10.1016/s0140-6736(11)61049-0

Che XH et al (2016) Dual inhibition of COX-2/5-LOX blocks colon cancer proliferation, migration and invasion in vitro. Oncol Rep 35:1680–1688. https://doi.org/10.3892/or.2015.4506

Chen EP, Smyth EM (2011) COX-2 and PGE2-dependent immunomodulation in breast cancer. Prostaglandins Other Lipid Mediators 96:14–20. https://doi.org/10.1016/j.prostaglandins.2011.08.005

Chikman B et al (2014) COX2 expression in high-grade breast cancer: evidence for prognostic significance in the subset of triple-negative breast cancer patients. Med Oncol 31:989. https://doi.org/10.1007/s12032-014-0989-1

Cianchi F et al (2006) Inhibition of 5-lipoxygenase by MK886 augments the antitumor activity of celecoxib in human colon cancer cells. Mol Cancer Ther 5:2716–2726. https://doi.org/10.1158/1535-7163.mct-06-0318

Cinatl J Jr, Vogel JU, Kotchekov R, Wilhem Doerr H (2004a) Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. FEMS Microbiol Rev 28:59–77. https://doi.org/10.1016/j.femsre.2003.07.005

Cinatl J, Scholz M, Kotchekov R, Vogel JU, Doerr HW (2004b) Molecular mechanisms of the modulatory effects of HCMV infection in tumor cell biology. Trends Mol Med 10:19–23

Cobbs CS et al (2002) Human cytomegalovirus infection and expression in human malignant glioma. J Neurooncol 8:2236–2244. https://doi.org/10.1016/j.jonné.2004.03.007

Dreyling KW, Hoppe U, Peskar BA, Morgenroth K, Kozusheck W, Peskar BM (1986) Leukotriene synthesis by human gastrointestinal tissues. Biochim Biophys Acta 878:184–193

Flavin DF (2007) A lipoxigenase inhibitor in breast cancer brain metastases. J Neurooncol 82:91–93. https://doi.org/10.1007/s11060-006-9248-4

Frascatoli G, Sinzer C (2014) Distinct properties of human cytomegalovirus strains and the appropriate choice of strains for particular studies. Methods Mol Biol 1119:29–46. https://doi.org/10.1007/978-1-62703-788-4_3

Gautam S, Roy S, Ansari MN, Saeed AS, Saraf SA, Kaithwas G (2017) DuCLOX-2/5 inhibition: a promising target for cancer chemoprevention. Breast Cancer 24:180–190. https://doi.org/10.1007/s12282-016-0723-2

Hammami R, Sumaida D, Zhang X, Das R, Jett M (2007) Control of the growth of human breast cancer cells in culture by manipulation of arachidonate metabolism. BMC Cancer 7:138. https://doi.org/10.1186/1471-2407-7-138

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674. https://doi.org/10.1016/j.cell.2011.02.013

Harkins L, Volk AL, Samanta M, Mikołajek I, Britt WJ, Bland KL, Cobbs CS (2002) Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet 360:1557–1563. https://doi.org/10.1016/S0140-6736(02)11524-8

Harkins LE et al (2010) Detection of human cytomegalovirus in normal and neoplastic breast epithelium. Herpesviridae 1:8. https://doi.org/10.1186/2042-4280-1-8

Harris RE, Beebe-Donk J, Alshafie GA (2006) Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer 6:27. https://doi.org/10.1186/1471-2407-6-27

Harris RE, Casto BC, Harris ZM (2014) Cyclooxygenase-2 and the inflammogenesis of breast cancer. World J Clin Oncol 5:677–692. https://doi.org/10.5306/wjco.v5.i4.677

Hennig R et al (2002) 5-Lipoxygenase and leukotriene B4 receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. Am J Pathol 161:421–428

Hooks JJ et al (2006) Human cytomegalovirus induced cyclooxygenase-2 in human renal epithelial cells augments viral replication through a prostaglandin pathway. Microbes Infect 8:2236–2244. https://doi.org/10.1016/j.micinf.2006.04.010

Howe LR (2007) Inflammation and breast cancer. Cyclooxygenase/Prostaglandin signaling and breast cancer. Breast Cancer Res 9:210. https://doi.org/10.1186/bcr1678

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90. https://doi.org/10.3322/caac.20107

Kotenko SV, Saccini S, Izotova LS, Mirochnitchenko OV, Pestka S (2000) Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci USA 97:1695–1700

Kumar A et al (2018) The human cytomegalovirus strain DB activates antiviral pathways in mammalian epithelial cells. EBioMedicine 30:167–183. https://doi.org/10.1016/j.ebiom.2018.03.015

Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C (2011) The detection of CMV pp65 and IE1 in glioblastoma multiforme. J Neurooncol 103:231–238. https://doi.org/10.1007/s11060-010-0383-6

Martinez-Gonzalez J, Badimon L (2007) Mechanisms underlying the cardiovascular effects of COX-inhibition: benefits and risks. Curr Pharm Des 13:2215–2227

Maussang D et al (2009) The human cytomegalovirus-encoded chemokine receptor US28 promotes angiogenesis and tumor formation via cyclooxygenase-2. Cancer Res 69:2861–2869. https://doi.org/10.1158/0008-5472.can-08-2487
Mosalpuria K et al (2014) Cyclooxygenase-2 expression in non-metastatic triple-negative breast cancer patients. Mol Clin Oncol 2:845–850. https://doi.org/10.3892/mco.2014.327
Na YR, Yoon YN, Son DJ, Seok SH (2013) Cyclooxygenase-2 inhibition blocks M2 macrophage differentiation and suppresses metastasis in murine breast cancer model. PLoS One 8:e63451. https://doi.org/10.1371/journal.pone.0063451
Petrucci N, Daly MB, Pal T (1993) BRCA1- and BRCA2-associated hereditary breast and ovarian cancer. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A (eds) GeneReviews(R). Seattle, WA
Poeckel D et al (2006) Inhibition of human 5-lipoxygenase and anti-neoplastic effects by 2-amino-1,4-benzoquinones. Med Chem 2:591–595
Qiu H, Straat K, Rahbar A, Wan M, Soderberg-Naucler C, Haeggstrom JZ (2008) Human CMV infection induces 5-lipoxygenase expression and leukotriene B4 production in vascular smooth muscle cells. J Exp Med 205:19–24. https://doi.org/10.1084/jem.20070201
Rahbar A et al (2015) Discordant humoral and cellular immune responses to Cytomegalovirus (CMV) in glioblastoma patients whose tumors are positive for CMV. Oncoimmunology 4:e982391. https://doi.org/10.4161/2162402x.2014.982391
Rahbar A et al (2017) Low expression of estrogen receptor-alpha and progesterone receptor in human breast cancer tissues is associated with high-grade human cytomegalovirus protein expression. Clin Breast Cancer 17(5):526–535. https://doi.org/10.1016/j.clbc.2017.04.013
Reeves MB, MacAry PA, Lehner PJ, Sissons JGP, Sinclair JH (2005) Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers. Proc Natl Acad Sci USA 102:4140–4145. https://doi.org/10.1073/pnas.0408994102
Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS (2003) High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol 170:998–1002. https://doi.org/10.1016/j.juro.2003.03.008
Schenk TE (2002) Inhibition of cyclooxygenase 2 blocks human cytomegalovirus replication. Proc Natl Acad Sci USA 99:3932–3937. https://doi.org/10.1073/pnas.020675099
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