Inflammation and Choline Metabolism are linked in Breast Cancer

I. Stasinopoulos[1], T. Shah[1], Y. Mironchik[1], B. Krishnamachary[1], and Z. M. Bhujwalla[1,2]

[1]JHU ICMIC Program, Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction: COX-1 and COX-2 are cytoplasmic enzymes that convert phospholipase A_2 (PLA_2)-mobilized AA into the lipid signal transduction molecules prostaglandins and thromboxanes [1]. One major product of the COX-2–catalyzed reaction is prostaglandin E_2 (PGE_2), an inflammatory mediator participating in several biological processes, including development, pain, immunity and angiogenesis [2], and cancer [3, 4]. Persistent expression of COX-2 has been linked to tumorigenesis and metastasis in solid tumors including breast where it is overexpressed in approximately 40% of cases [5]. Silencing of COX-2, by a specific short hairpin RNAi molecule, reduced tumor onset, inhibited extrapulmonary metastasis, reduced angiogenesis and the secretion of [H+] and lactate, by the highly metastatic poorly differentiated breast cancer cells MDA-MB-231 [6, 7].

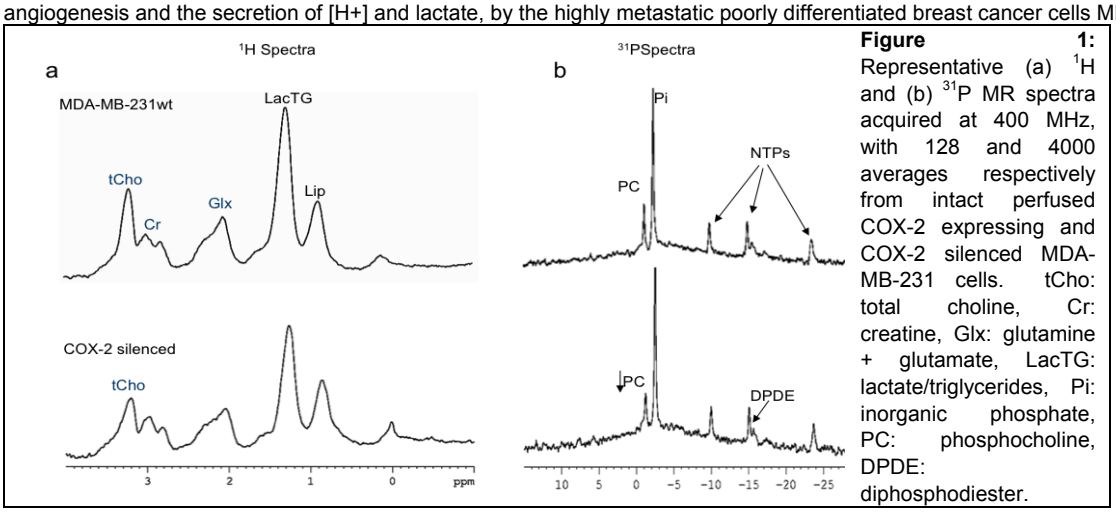

Figure 1: Representative (a) ^{1}H and (b) ^{31}P MR spectra acquired at 400 MHz, with 128 and 4000 averages respectively from intact perfused COX-2 expressing and COX-2 silenced MDA-MB-231 cells. tCho: total choline, Cr: creatine, Glx: glutamine + glutamate, LacTG: lactate/triglycerides, Pi: inorganic phosphate, PC: phosphocholine, DPDE: diphosphodiester.

We previously observed that choline metabolism is altered following pharmacological inhibition with indomethacin, a nonspecific COX inhibitor [8]. Similarly, COX-2 specific silencing in MDA-MB-231 human breast cancer cells stably expressing COX-2 shRNA reduces total choline and phosphocholine (PC) (Figure 1). Here we have further characterized the molecular mechanism underlying this alteration and confirmed a close association between COX-2 and choline kinase (Chk), the enzyme that converts choline to PC. Since total choline and PC are being evaluated as biomarkers of cancer [9], it is important to understand the role of inflammation in the elevated total choline/PC phenotype.

Methods: MDA-MB-231 cells (~ $2x10^6$) were treated with IL-1β (10 ng/μl) for the times indicated. At a concentration of 10 ng/μl, interleukin-1β (IL-1β) induces COX-2 expression within 2 - 6 h of treatment. To further understand the relationship between Chk and COX-2, we treated MDA-MB-231 clone 2 cells that have COX-2 silenced (clone 2) with PGE_2. Clone 2 cells (~ $2x10^6$) were treated with PGE_2 (50 nM) for the times indicated.

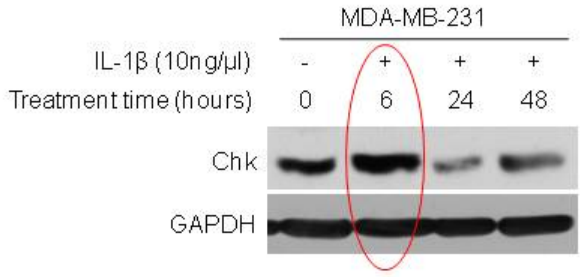

Figure 2a: Chk expression levels in untreated MDA-MB-231 cells and cells treated with IL-1β (10 ng/μl) for 6, 24 and 48 h. An increase of Chk expression was observed at 6 h (outlined in red) that decreased by 24 h.

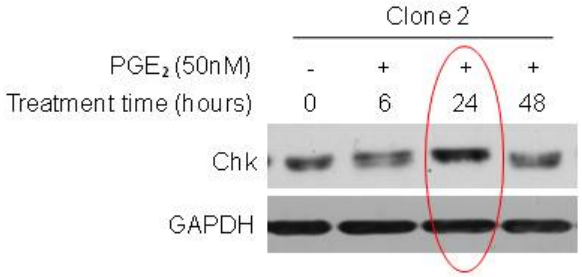

Figure 2b: Chk expression levels in MDA-MB-231 clone 2 cells that stably express shRNA against COX-2 and have low basal and inducible COX-2 and PGE_2 levels, following **'add-back'** of PGE_2. Here **'add-back'** of PGE_2 in clone 2 cells resulted in an increase of Chk at 24 h (outlined in red).

Results and Discussion: We observed an approximately two-fold increase of Chk within 6 h of treatment of MDA-MB-231 cells, with IL-1β as shown in Figure 2a. Interestingly, this was followed by a decrease at 24 h. We have previously observed significant HIF-1 stabilization in response to inflammatory stimuli such as treatment with IL-1β (10 ng/ml), which was abolished in COX-2 silenced cells [10]. Our previously published studies have identified HIF-1 binding sites in the Chk promoter region [11]. Induction of HIF-1 and its binding to hypoxia response elements in the Chk promoter to increase Chk transcription may be one possible mechanism by which Chk expression increases with induction of COX-2. As shown in Figure 2b, when PGE_2 was added back to MDA-MB-231 clone 2 cells, an increase of Chk was observed at 24 h that returned to basal levels at 48 h. Collectively, Figures 1 and 2 provide direct evidence for COX-2 – mediated changes in Chk and choline metabolism. In a complex disease such as cancer, it is important to understand the network of pathways that participate following downregulation of a specific target as these provide novel opportunities to target in concert to achieve improved control and minimize compensatory responses that allow the cell to survive or adapt.

Acknowledgements: This work was supported by NIH R01 CA82337 and P50 CA103175.

References: 1. van der Donk WA *et al.*, Biochemistry. 2002; 2. Smith WL *et al.*, Annu Rev Biochem. 2000; 3. Howe LR. Breast Cancer Res. 2007; 4. Singh-Ranger G *et al.*, 2008; 5. Ristimaki A *et al.*, Cancer Res. 2002; 6. Stasinopoulos I *et al.*, Neoplasia. 2008; 7. Stasinopoulos I *et al.*, Mol Cancer Res. 2007; 8. Glunde K *et al.*, Magn Reson Med. 2002; 9. Haddadin IS *et al.*, NMR Biomed. 2009; 10. Stasinopoulos I *et al.*, Cancer Biol Ther. 2009; 11. Glunde K *et al.*, Cancer Res. 2008.