Inflammation and Choline Metabolism are linked in Breast Cancer

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 RNA 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]. 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/ul) for the times indicated. At a concentration of 10 ng/ul, 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$^5$) were treated with PGE$_2$ (50 nM) for the times indicated.

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 element 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.