Cyclooxygenase-2 mediates changes in the extracellular matrix in triple negative breast cancer

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Introduction: Triple (estrogen receptor, progesterone receptor, HER2-neu) negative breast cancer (TNBC) is one of the most lethal forms of breast cancer and pathological response does not translate to better survival in TNBC patients. We have observed increased expression of COX-2, in several TNBC cells that were also metastatic. There is increasing evidence to suggest that high density of collagen I fibers in the ECM is a predictor of increased metastasis [1]. Cancer cells have been observed to migrate along these fibers during the metastatic journey. We previously observed that downregulation of COX-2 significantly reduces the expression of degradative enzymes such as matrix metalloproteinase 1 (MMP1) and alters the expression of ECM components such as hyaluronan and lumican that play a role in intra-fibrillar collagen spacing [2]. It is therefore highly likely that COX-2 can modify the ECM, but studies relating ECM structure and function with COX-2 are lacking. The ECM plays a major role in drug delivery, invasion and metastasis. To the best of our knowledge the relationships between COX-2, alteration of collagen fibers and macromolecular transport through the ECM have not been examined in TNBC.

Methods: Tumors derived from the triple negative human breast cancer cell line MDA-MB-231 and its clone (Clone 13) with different basal and inducible COX-2 expression levels (**Table 1**) were studied *in vivo* following orthotopic implantation ($2x10^6$ cells in Hanks balanced salt solution) in the right upper thoracic mammary fat pad of female SCID mice. Tumors derived from these cells were used to investigate the relationship between COX-2 expression, interstitial fluid transport using MRI and collagen fiber density and volume using second harmonic generation (SHG) microscopy. MRI was performed once tumor volumes were approximately 400-500 mm^3. Interstitial transport parameters were measured from quantitative T_1 maps obtained before and following intravenous administration of the contrast agent albumin-GdDTPA (500 mg/kg dose) on a 4.7T Bruker spectrometer. Images were acquired in two "phases" corresponding to the biphasic kinetics of the MMCA. The "early phase" images obtained over the initial 30 min. were used to characterize the tumor vasculature. Since drainage of macromolecules in and around tumors either by convection or by the lymphatics is a slow event, the second block of MR data was acquired up to 140 min post contrast and was used to characterize interstitial transport. Interstitial transport parameters calculated included number of draining and pooling voxels, draining and pooling rates, and volumes as previously described in [3]. SHG microscopy of tissue slices was performed using a 25× lens on a Zeiss 710 LSM NLO confocal microscope system equipped with a 680–1080 nm tunable Coherent Chameleon Vision II laser with automated pre-compensation and fast scanning at 40 nm/s. 3D image stacks were acquired from various fields of view (FOVs). Collagen I fibers were imaged with incident laser light of 880 nm, detected at 410-470 nm. Collagen I fiber distribution was visualized and characterized using an in-house 3D analysis software developed to quantify fiber distance distributions and fiber volumes [4].

Table 1: Prostaglandin E2 (PGE_2) levels secreted by MDA-MB-231 cells and Clone 13 cells stably expressing COX-2 shRNA

Cells	MDA-MB-231	Clone 13
PGE_2 levels (pg/ml) without IL-1β	232.1	96.5
PGE_2 levels (pg/ml) with 10 ng/ml IL-1β	13992.8	1152.9

Results and Discussion: Significant differences in the ECM structure and function were evident between high COX-2 expressing MDA-MB-231 tumors compared to Clone 13 tumors with low COX-2 expression. The representative maps in **Figure 1a** demonstrate the increase of vascular volume, decrease of draining voxels, increase of pooling voxels, and decrease of collagen fibers following a reduction of COX-2 expression. Results obtained from three mice in each group are summarized in **Figures 2a-d**. Values represent mean +/- SEM. Collectively these data provide direct evidence for COX-2 – mediated changes in the ECM. A recent study demonstrated that tumor cancer cell interaction with collagen 1 fibers induces COX-2 and that cells close to high-density collagen 1 have high COX-2 [5]. Here, for the first time, we have shown that dowregulating COX-2 in TNBC cells profoundly impacts collagen 1 fiber density and volume and alters macromolecular transport. Understanding the role of COX-2 in TNBC and its impact on the ECM and macromolecular transport may provide new insights into preventing relapse and metastasis.

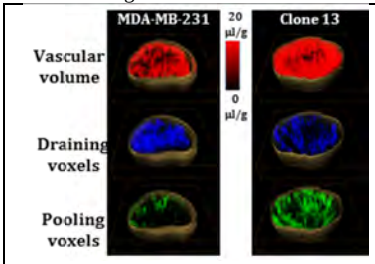

Figure 1a: 3D visualization of representative maps of vascular volume (in red), draining voxels (in blue) and pooling voxels (in green) from an MDA-MB-231 tumor and a Clone 13 tumor.

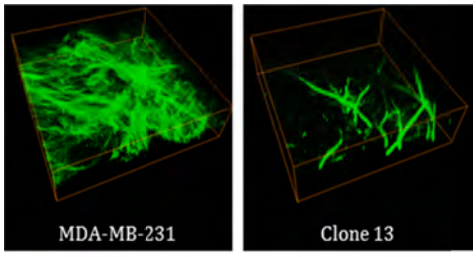

Figure 1b: 3D Collagen fiber distribution from a representative FOV from a MDA-MB-231, (339.41 µmx41µm×339.41µmx41µm× 85µm) and Clone 13 tumor, 339.41µmx41µm× 339.41µmx41µm×105µm) FOV.

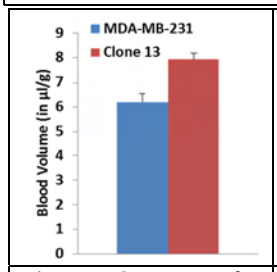

Figure 2a: Vascular volume in Clone 13 tumors was significantly higher (*p-value*=0.016) compared to MDA-MB-231 tumors.

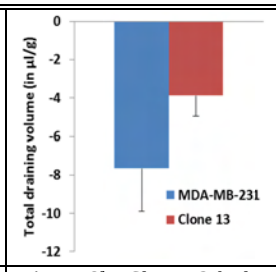

Figure 2b: Clone 13 had a trend towards lower draining voxels compared to MDA-MB-231 tumors (*p-value*= 0.15, n=3).

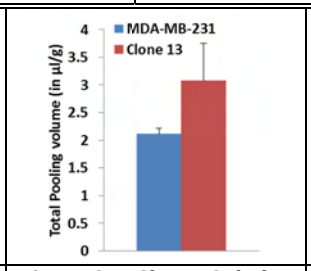

Figure 2c: Clone 13 had a trend towards higher pooling voxels compared to MDA-MB-231 tumors (*p-value*= 0.18, n=3).

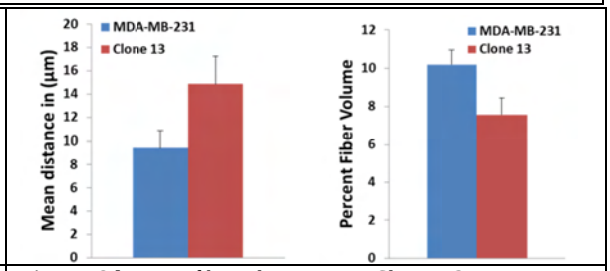

Figure 2d: Inter-fiber distance in Clone 13 tumors was significantly higher compared to MDA-MB-231 tumors, *p*= 0.042 (graph on left); Clone 13 tumors had significantly lower percentage of collagen fiber volume *versus* MDA-MB-231 tumors, *p*= 0.024 (graph on right)

Acknowledgements: This work was supported by NIH R01 CA82337 and P50 CA103175. **References:** 1. Conklin MW *et al.*, Am J Pathol. 2011; 2. Stasinopoulos I *et al.*, Mol Cancer Res, 2007; 3. Pathak AP *et al.*, Cancer Res, 2006; 4. Kakkad SM *et al.*, Neoplasia. 2010. 5. Lyons *et al.*, Nature Med., 2011.