

Cyclooxygenase-2 Silencing of MDA-MB-231 Breast Cancer Cells Reduces Invasion and Alters Metabolism

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Introduction: COX-2 is a cytoplasmic enzyme responsible for the conversion of arachidonic acid to active lipid mediators whose expression is induced by pro-inflammatory cytokines such as IL-1 β . COX-2 is upregulated in different cancers such as colon, prostate, and breast. We have previously demonstrated that silencing COX-2, using stably expressed COX-2-specific short hairpin RNA, in the poorly differentiated and highly metastatic human breast cancer cell line MDA-MB-231 delayed tumor formation and inhibited extrapulmonary metastasis in an experimental model of metastasis (1).

Material and Methods: We have dynamically tracked invasion and metabolism of COX-2-silenced MDA-MB-231 cells using an MR compatible cell perfusion assay, the Metabolic Boyden Chamber (MBC). Parental MDA-MB-231 cells, Clone 2 cells (COX-2-silenced cells from a single clone), and cells pooled (Pooled) from four individual clones were selected for these studies.

MR-compatible Cell Invasion Assay: For the MR cell perfusion system studies, cells were seeded on Biosilon beads (Nunc, Denmark) beads at a cell density of 1.5 X10⁶ cells per 0.5 ml of microcarriers three days before the experiments in non-cell culture petri dishes, and grown adherently to beads to approximately 70% confluence. The sample consisted of different layers containing filter material, cell covered beads, a home-built chamber filled with the extracellular matrix (ECM), Matrigel®, and perfluorocarbon-doped alginate beads to monitor oxygenation, as previously described (2).

Results & Discussion: Figure 1a shows representative ¹H MR images of ECM gel degradation acquired over 48 h that demonstrate significant degradation of ECM gel by MDA-MB-231 cells. In contrast, Pooled and Clone 2 COX-2-silenced cells showed reduced degradation of ECM relative to parental cells. For statistical analysis data from Pooled (n=2) and Clone 2 cells (n=2) were pooled together. Quantitative time-dependent invasion indices I₀ obtained from diffusion weighted 1D ¹H profiles of intracellular water profiles demonstrated significantly reduced invasion by COX-2-silenced cells (p < 0.03) compared to parental cells (Figure 1b).

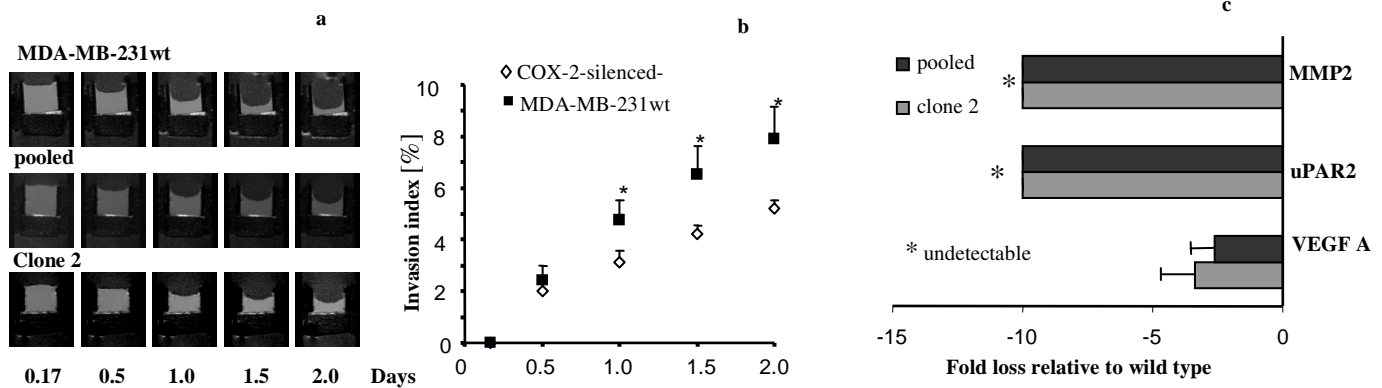


Figure 1: (a) Representative T₁-weighted ¹H MR images showing degradation of ECM gel by parental MDA-MB-231 cells and reduced degradation of ECM gel by COX-2-silenced Pooled and Clone 2 cells. (b) Quantitative time-dependent invasion indices I(t) were obtained from intracellular diffusion-weighted water profiles at various time points. Values are mean \pm SD (n = 4; * p < 0.03). (c) qPCR of differences in expression of invasion related genes (n=3).

Quantitative PCR showed reduced expression of VEGFA and undetectable levels of uPAR2 and MMP2, genes involved in invasion, in COX-2-silenced cells compared to parental cells (Figure 1c). Reduced total choline (tCho), phosphocholine (PC) levels and lactate (Lac) were detected in ¹H MR spectra of COX-2-silenced cells relative to parental MDA-MB-231 cells. Figure 2 shows quantitative data summarized from ¹H MR spectra of COX-2-silenced cells and MDA-MB-231 parental cells over the course of two days. Levels of tCho (p < 0.03), PC (p < 0.03) and Lac (p < 0.06) in COX-2-silenced cells were significantly lower than parental cells at the time points studied (Figure 2 a-c). We also determined the choline kinase (Chk) expression through immunoblotting and found a lower expression of Chk in COX-2-silenced cells compared to COX-2 expressing parental cells (data not shown).

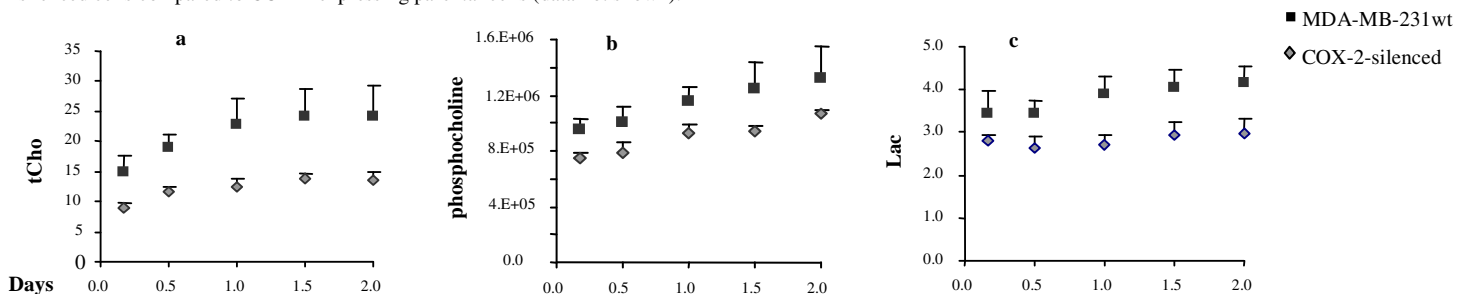


Figure 2: Quantification of ¹H and ³¹P MR spectra identified significant differences in the amounts of tCho (a) PC (b) and Lac (c) between COX-2-silenced and COX-2 containing cells. Values are means \pm SD (n = 4; p < 0.03 for tCho, p < 0.03 for PC and p < 0.06 for Lac).

The data presented here demonstrate that COX-2-silenced MDA-MB-231 cells acquired a significantly reduced ability to degrade and invade the ECM Matrigel® relative to parental cells. Reduced invasion by COX-2-silenced cells can be attributed to reduced expression of genes involved in invasion such as MMP-2, VEGF-A, and uPAR. The reduction of Chk activity and total choline in COX-2-silenced cells is consistent with previous observations that a reduction in choline kinase activity is observed in less malignant cells (3).

Conclusion: COX-2-silenced MDA-MB-231 breast cancer cells were significantly less invasive and acquired distinctive metabolic characteristics typical of less aggressive breast cancer cells. These data support the use of COX-2 inhibitors in reducing or preventing breast cancer metastasis.

References: (1) Stasinopoulos I *et al.* Mol Cancer Res 2007; 5: 435–42. (2) Ackerstaff E *et al.* Neoplasia. 2007, 9: 222-35. (3) Glunde K *et al.* Cancer Res. 2005 65:110434-43.

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