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2014

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**Recommended Citation**
Algaly, Fahad; Hand, Julia; and Sevigny, Mary B., "Glycosylation of cyclooxygenase-2 (COX-2) influences the migratory and invasive potential of cells" (2014). *Natural Sciences and Mathematics | Faculty Research Posters*. 2.
https://scholar.dominican.edu/natural-sciences-and-mathematics-faculty-research-posters/2

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# Glycosylation of cyclooxygenase-2 (COX-2) influences the migratory and invasive potential of cells

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## ABSTRACT

**Prostaglandins are bioactive lipids involved in many physiological functions such as maintenance of the cardiovascular, immune, renal, and central nervous systems. They also play a role in certain diseases like arthritis, cancer, and Alzheimer's. Cyclooxygenase-2 (COX-2) is the enzyme that catalyzes the initial rate-limiting step in the pathway that converts arachidonic acid to prostaglandins. COX-2 exists as two glycoforms with the molecular weights of 72 and 74 kDa, the latter resulting from the addition of a high mannose chain to the Asn580 residue ~50% of the time. The over-expression of COX-2 is believed to be linked to cancer progression and specifically appears to promote the metastatic phenotype. The objective of this study is to determine the effect of the variable glycosylation of COX-2 at $Asn^{580}$ on the migratory and invasive potential of cells. COS-1 cells and the breast cancer cell line MCF7 were first transfected with either the wild type or $Asn^{580}$-mutant human COX-2 gene. Boyden chambers were used to determine the ability of transfected cells to migrate through the membrane, approximately $5x10^4$ cells were plated onto the chambers, and cells were incubated for 16-18 h. Cells were then fixed, stained, visualized and counted. In a previous study, our lab showed that COS-1 cells transfected with the $Asn^{580}$-mutant COX-2 gene migrated faster through the membrane. In this current study, COS-1 cells transfected with the $Asn^{580}$-mutant COX-2 gene also had a greater invasive potential; however, MCF7 cells transfected with the wild-type human COX-2 gene migrated faster and also had a greater tendency to invade. The results indicate that the ability of this additional or the lack of glycosylation of COX-2 at $Asn^{580}$ to either enhance or inhibit the migratory and invasive potential of cells depends greatly on cell type. To confirm this, future studies will be carried out to determine the effect of COX-2 glycosylation on the invasive and the migratory potential of PC-3 and T-47D cancer cell lines.**

## INTRODUCTION

COX-2 is an enzyme expressed in most cells. It's the enzyme involved in the formation of prostaglandins from arachidonic acid. Prostaglandins (PGs) are a group of lipids that impact our body both positively and negatively. They improve circulation and nerve formation, increase T-cell formation, regulate blood pressure, and regulate many other physiological functions. The overexpression of COX-2 leads to the over production of PGs– like $PGE_2$– and in turn leads to a broad array of diseases like inflammation, Alzheimer's, rheumatoid arthritis and many cancers such as prostate, colon and breast cancer. Of all the prostaglandins, $PGE_2$ is most implicated in cancer progression and the development of more advanced phenotypes such as metastasis.

The COX-2 protein is located in the membrane of the endoplasmic reticulum and the nuclear envelope. The enzyme has five potential N-linked glycosylation sites – $Asn^{53}$, $Asn^{130}$, and $Asn^{396}$ are always glycosylated, $Asn^{592}$ is never glycosylated, and $Asn^{580}$ is glycosylated 50% of the time. This latter variable glycosylation site produces two glycoforms of 72 and 74 kDa.

Our lab has previously shown that this additional glycosylation plays a role in the turnover of the COX-2 enzyme and negatively affects the efficacy of various COX-2 inhibitors. Since other researchers have shown that overexpression of COX-2 in cells leads to a more enhanced metastatic phenotype, our lab is interested in determining if the glycosylation of COX-2 affects this as well. Therefore, the purpose of this study is to find out if the metastatic potential of COX-2-expressing cells depends upon which glycoform(s) is(are) expressed.

## METHODS

### *Transfection of COS-1 and MCF-7 cells with the COX-2 gene*

The transfection of cells with either the wild-type human COX-2 gene or the $Asn^{580}$-mutant human COX-2 gene was carried out in 5% FBS/DMEM using *Trans*It-LT1 transfection reagent. Control cells were transfected with an empty pcDNA3.1 plasmid from Invitrogen. Transfection efficiency was confirmed by co-transfection with pEGFP-1 followed by fluorescent microscopy detection. Expression of the COX-2 glycoforms was verified via Western blots.

### Analysis of cell migration and invasion

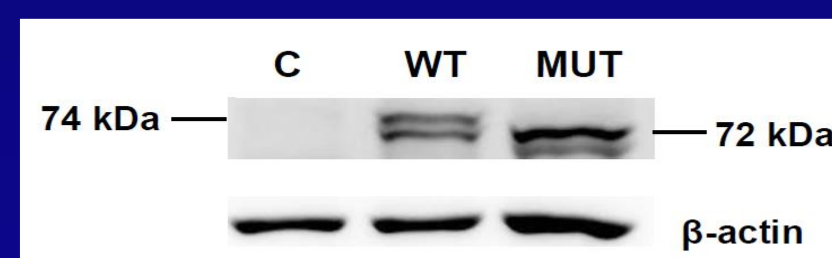
Transfected cells at a density of 5 x $10^4$ cells per well were plated onto Boyden chambers with 8 µm pore size or onto modified Boyden chambers with an extra layer of matrigel to test cells' migratory and invasive potential, respectively. Cells were then incubated at 37 °C. After 12-18 hours of incubation, cells were fixed in 10% phosphate buffered formalin. Crystal violet was used to stain cells, and then the chambers were placed in distilled $H_2O$ to rinse off any excess crystal violet. Cotton swabs were used to gently clean the insides of the Boyden chambers. After drying, the membranes were viewed under a light microscope using the computer program MOTIC to view and capture the images. The pictures were used to count how many cells migrated or invaded across the membranes.

### Expression level of prostaglandin $E_2$ ($PGE_2$) receptors

Total RNA was isolated from the transfected MCF-7 cells using TRI Reagent following the manufacturer's protocol. After isolation, first strand cDNA was made, and PCR was carried out to determine the expression levels of the four $PGE_2$ receptors– EP1, EP2, EP3, and EP4. ß-actin gene was used as house-keeping gene for normalization.

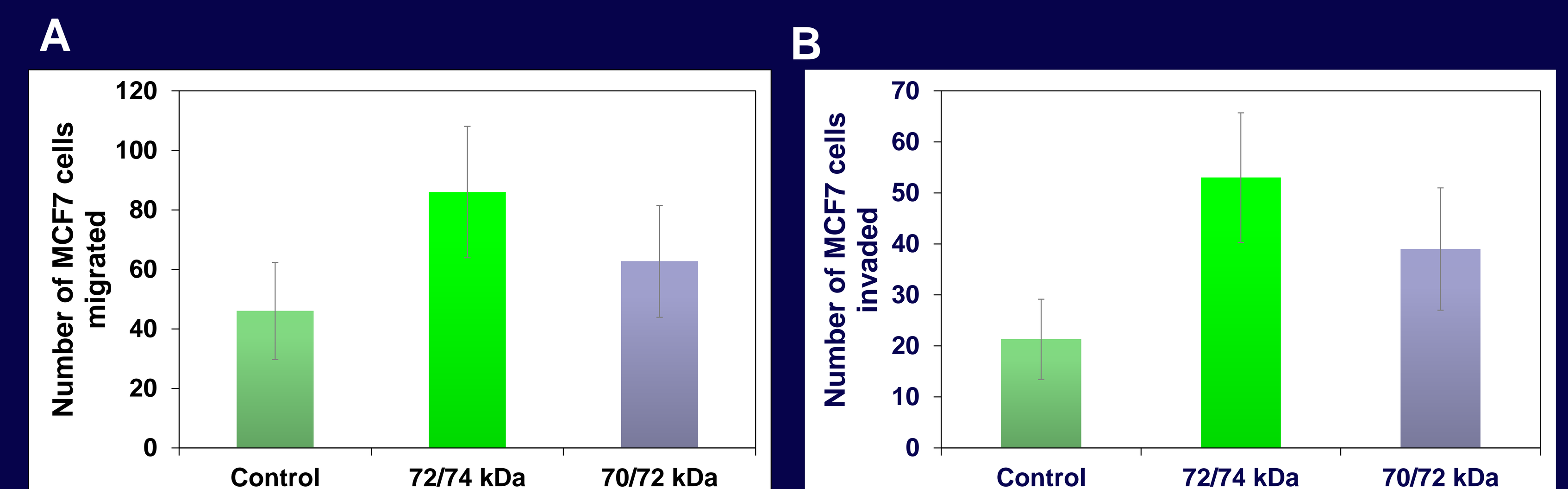
## RESULTS

### Figure 1



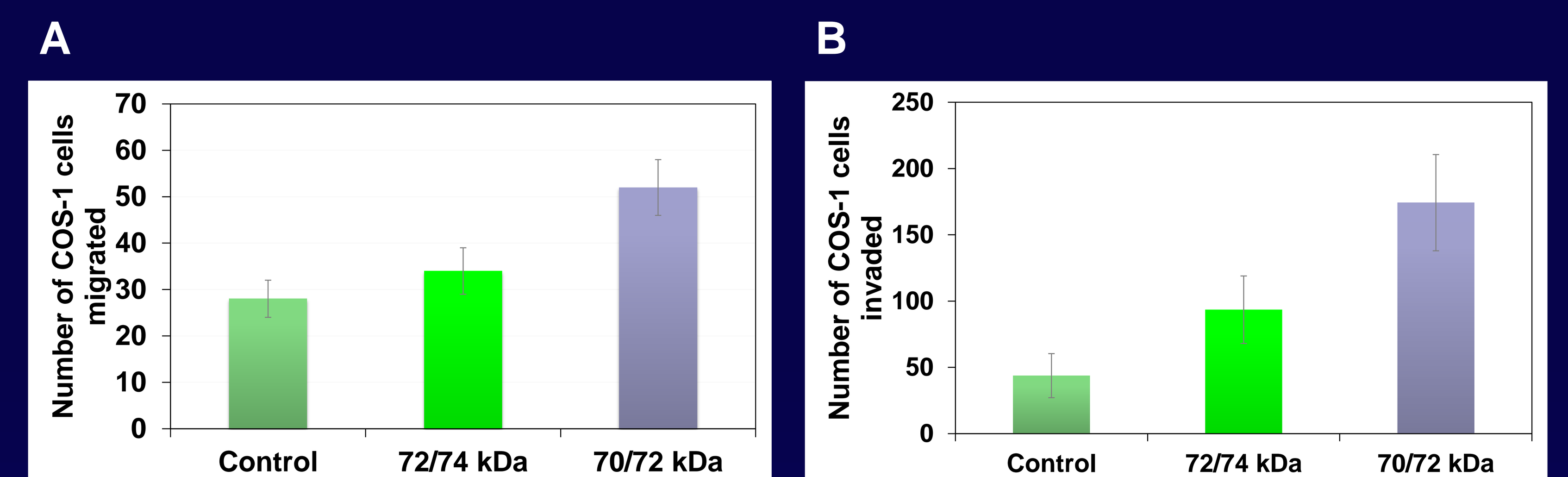

**Western blot data showing expression of the COX-2 glycoforms**. C = control, COS-1 cells transfected with empty plasmid; WT = COS-1 cells transfected with the wild-type COX-2 gene; MUT = COS-1 cells transfected with the mutant gene. Cells transfected with the wild-type gene expressed the 72 and 74 kDa glycoforms whereas cells transfected with the $Asn^{580}$-mutant gene expressed the 72 and a "new" 70 kDa glycoform.

### Figure 2



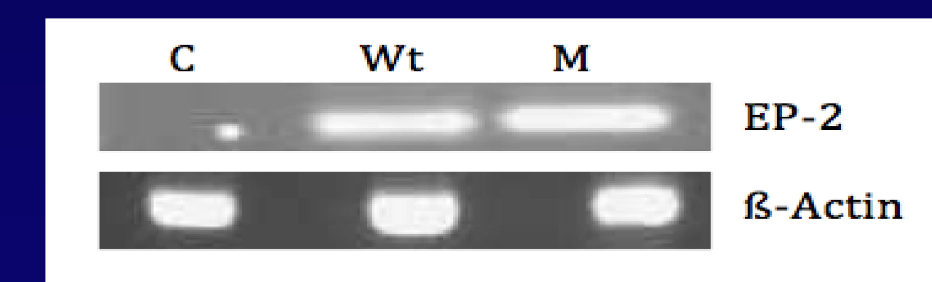

**Effect of glycosylation of COX-2 at $Asn^{580}$ on the migratory (A) and invasive (B) abilities of MCF-7 cells.** Control = MCF-7 cells transfected with empty plasmid; 72/74 kDa = MCF-7 cells expressing the 72 kDa and 74 kDa COX-2 glycoforms; 70/72 kDa = MCF-7 cells expressing the 70 and 72 kDa COX-2 glycoforms. Results are averages ± SEM; n = 3 - 4 independent experiments.

### Figure 3




**Effect of glycosylation of COX-2 at $Asn^{580}$ on the migratory (A) and invasive (B) abilities of COS-1 cells.** Control = COS-1 cells transfected with empty plasmid; 72/74 kDa = COS-1 cells expressing the 72 kDa and 74 kDa COX-2 glycoforms; 70/72 kDa = COS-1 cells expressing the 70 and 72 kDa COX-2 glycoforms. Results are average ± SEM; n = 3 - 4 independent experiments.

### Figure 4




**RT-PCR detection of EP-2 receptors in MCF7 cells.** C = MCF-7 cells transfected with empty plasmid; Wt = MCF-7 cells transfected with the wild-type COX-2 gene; M = MCF-7 cells transfected with the $Asn^{580}$- mutant COX-2 gene.

## CONCLUSIONS

- **Lack of glycosylation of COX-2 at $Asn^{580}$ enhances the migratory and invasive potential of COS-1 cells.**
- **However, the addition of glycosylation at $Asn^{580}$ enhances the migratory and invasive ability of MCF-7 cells.**
- **The prostaglandin $E_2$ receptor, EP2 is up-regulated in MCF-7 cells overexpressing COX-2, regardless of its glycosylation state. Result may partially explain the molecular mechanism behind COX-2's role in metastasis.**