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Glycosylation of cyclooxygenase-2 (COX-2) influences the migratory potential of COS-1 cells

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ABSTRACT

A cancer cell's most threatening property is its ability to metastasize or detach from the primary tumor and migrate to other locations in the body. Previous studies have shown that overexpression of the enzyme cyclooxygenase-2 (COX-2) can increase the metastatic potential of several cell types. COX-2 is the rate-limiting enzyme in the prostanoid biosynthesis pathway, converting arachidonic acid to prostaglandin H_2, an important signaling molecule in the body. Glycosylation of COX-2 at the amino acid site Asn^{580} occurs about 50% of the time, and this results in two forms of the enzyme with molecular weights 72 and 74kDa. The purpose of this study was to investigate the impact of glycosylation of COX-2 at the Asn^{580} site on the metastatic potential of cells. COS-1 cells were first transfected with either an Asn^{580}-mutant human COX-2 gene or the wild-type human COX-2 gene. A cell migration assay was then carried out on these two groups of cells. Briefly, $5x10^4$ cells were plated onto the membrane of a Boyden Chamber, and cells were incubated for 12 hours. Cells that migrated to the underside of the membrane were fixed, stained, visualized via light microscopy, and counted. Our results revealed that cells transfected with the Asn^{580}-mutant gene migrated faster through the membrane. This indicates that a lack of glycosylation at the Asn^{580} site of the COX-2 enzyme may lead to an enhanced metastatic potential in cells. Future studies will analyze the effect of variable COX-2 glycoform expression on the migratory potential of tumor cell lines such as MCF-7 and T-47D.

INTRODUCTION

The COX-2 enzyme is found in most vertebrate tissues. COX-2 catalyzes the synthesis of prostaglandin H_2 (PGH_2) from the omega-6 fatty acid arachidonic acid. PGH_2 is then quickly converted into other prostaglandins by downstream prostaglandin synthases. These various prostaglandin isomers are capable of initiating inflammation, fever, pain sensation, and vasodilation and also play roles in maintenance of renal function, bone metabolism, and pregnancy. Known primarily for its role in inflammatory responses, overexpression of COX-2 has also been found to have a role in Alzheimer's disease, rheumatoid arthritis, and many cancers such as colorectal and breast cancer .

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 cells with the COX-2 gene

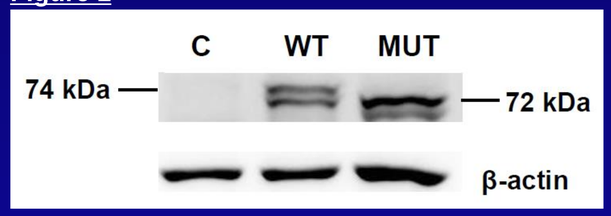
The transfection of COS-1 cells with either the wild-type human COX-2 gene or the Asn^{580}-mutant human COX-2 gene was carried out in 5% FBS/DMEM using *Trans*It-LT1 transfection reagent from Mirus Bio. Control COS-1 cells were transfected with an empty pcDNA3.1 plasmid from Invitrogen. Transfection 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.

Observation of COS-1 cell migration

Transfected cells at a cell density of 5×10^4 cells per well were plated into Boyden chambers with 8 µm pore size and incubated at 37°C, 5% CO_2. After 12 hours of incubation, cells were fixed in 10% phosphate buffered formalin for at least 15 minutes. Crystal violet was used to stain each chamber for 10 minutes, 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, and the chambers were allowed to air dry for 1 hour to remove any excess water. After drying, the membranes were viewed under a light microscope using the computer program MOTIC to view and capture the images as jpg files. The pictures were used to count how many cells migrated across the membranes.

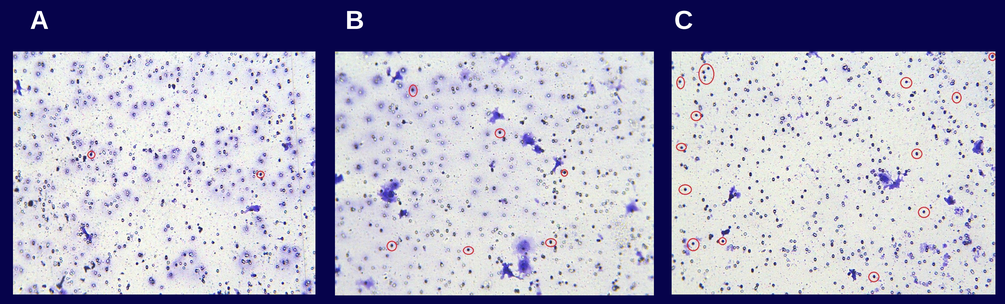
RESULTS

Figure 1



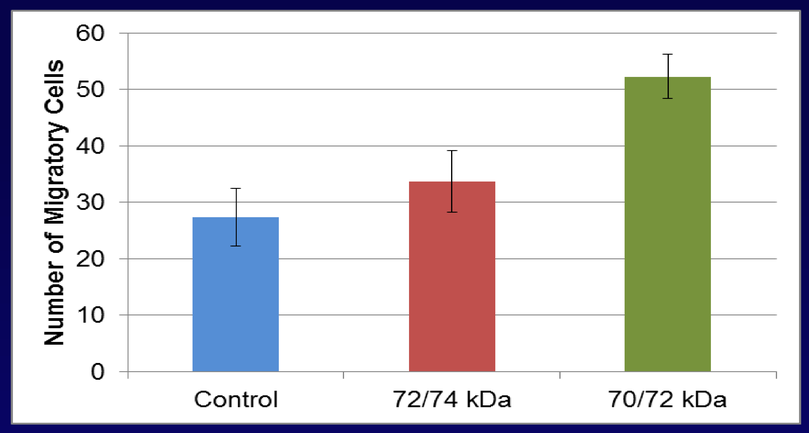
Western blot data showing expression of the COX-2 glycoforms. C = control, COS-1 cells transfected with empty plasmid; WT = COS-1 cells transfected with the wild-type COX-2 gene; MUT = COS-1 cells transfected with the mutant gene. Cells transfected with the wild-type gene expressed the 72 and 74 kDa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

Figure 2



Sample membranes from a COS-1 cell migration assay. A) Control COS-1 cells transfected with empty pcDNA3.1 plasmid. **B)** COS-1 cells expressing the 72/74 kDa COX-2 glycoforms. **C)** COS-1 cells expressing the 70/72 kDa COX-2 glycoforms. Red circles show cells that are migrating through the membrane of the Boyden chamber. The cell fills the entirety of the pore with a strong, purple color. Images of the Boyden chamber membranes were taken at 200x magnification under a light microscope using Motic software.

Figure 3



Effect of glycosylation of COX-2 at Asn^{580} on the migratory ability of COS-1 cells. Control, COS-1 cells transfected with empty plasmid; 72/74 kDa, COS-1 cells expressing the 72 and 74 kDa COX-2 glycoforms; 70/72 kDa, COS-1 cells expressing the 70 and 72 kDa COX-2 glycoforms. Results are averages ±SEM; n = 3 independent experiments.

CONCLUSION

The COS-1 cells expressing the 70/72 kDa COX-2 glycoforms had a greater tendency to migrate across the membrane, indicating that the lack of glycosylation of COX-2 at Asn^{580} enhances the migratory potential of COS-1 cells.

FUTURE STUDIES

- **Observe the effect of variable COX-2 glycoform expression on the migratory potential of tumor cell lines such as MCF-7 and T-47D.**
- **Use 2D gel electrophoresis to observe any differences in protein expression between the 70/72kDa and 72/74kDa COX-2-expressing cells.**
- **Determine if this glycosylation affects the invasive potential of cells.**