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Effect of Cyclooxygenase-2 Glycosylation on Downstream Expression of E-Cadherin and β-Catenin in MCF-7 Breast Cancer Cells

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INTRODUCTION & OBJECTIVE

Cyclooxygenase-2 (COX-2) is an enzyme that helps catalyze the formation of prostaglandins, which promote inflammation, pain, and fever and maintain other normal physiological functions throughout the body[1]. However, the overexpression of COX-2 has been found to play a role in various diseases including breast cancer[1]. COX-2 exists as two major glycoforms— 72 kDa and 74 kDa— due to the glycosylation site Asn^{580} which is glycosylated 50% of the time[2]. Past studies from our lab have shown that this glycosylation regulates COX-2 protein turnover in the cell[3] (**Figure 1**). The proteins E-cadherin— a tumor suppressor— and β-catenin— a tumor driver— can be regulated by COX-2 activity[4].

The purpose of this study is to determine if the glycosylation of COX-2 at Asn^{580} affects the downstream expression of E-cadherin and β-catenin and subsequent migratory potential in MCF-7 breast cancer cells.

EXPERIMENTAL PROCEDURES

Transfection of 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 using *Trans*It-LT1 transfection reagent from Mirus Bio. 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

Transfected cells at a density of 5 x 10^4 cells per well were plated onto Boyden chambers with 8 µm pore size to test cells' migratory potential. Cells were then incubated at 37 °C for 22 hours. After incubation, cells were fixed in 10% phosphate buffered formalin and then stained with crystal violet. After drying, the membranes were viewed under a light microscope using the computer program MOTIC to view and capture the cell images which were then used to count how many cells migrated to the underside of the membrane.

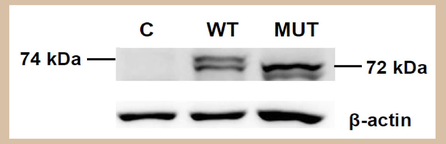
[1]Hinz and Brune (2001) *Perspectives in Pharmacology*, **300**(2): 367
[2]Otto et *al.* (1993) *The Journal of Biological Chemistry*, **268**(24): 18234
[3]Sevigny et *al.* (2006) *FEBS Letters*, **580**: 6533
[4]Jang et al. (2010) *Virchows Archiv*, **457**(3): 319

Expression levels of E-cadherin and ß-catenin

- *Semi-quantitative RT-PCR*: 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 using custom primers from IDT to determine the expression levels of E-cadherin and ß-catenin. ß-actin was used as a housekeeping gene for normalization.
- *Western blotting*: Whole cell lysates prepared from transfected MCF-7 cells were subjected to SDS-PAGE using Bio-Rad Mini-PROTEAN® TGX™ pre-made gels. Proteins were transferred to a PVDF membrane using the Bio-Rad Trans-Blot® Turbo™ Blotting system following the manufacturer's directions. Blots were subjected to immunostaining using either the anti-E-cadherin monoclonal antibody or the anti-ß-catenin polyclonal rabbit antibody (Santa Cruz Biotechnology). ß-actin served as a housekeeping protein for normalization (monoclonal antibody from Sigma).

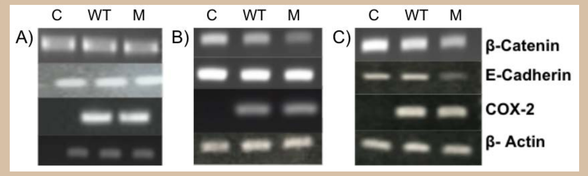
RESULTS

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.

Figure 2

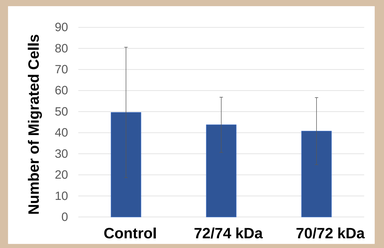


Effect of glycosylation of COX-2 at Asn^{580} on the transcriptional expression of E-cadherin and β-catenin in MCF-7 cells. C = control, MCF-7 cells transfected with empty plasmid; WT = MCF-7 cells transfected with wild-type COX-2 gene; and M = MCF-7 cells transfected with Asn^{580}-mutant COX-2 gene. The mRNA expression levels of β-catenin, E-cadherin, COX-2, and β-actin ("housekeeping" gene), were analyzed via RT-PCR at 24 (**A**), 48 (**B**), and 72 hours (**C**) post transfection.

Figure 3

Effect of COX-2 glycosylation at Asn^{580} on the Protein Expression of E-cadherin and β-catenin in MCF-7 cells. C = control, MCF-7 cells transfected with empty plasmid; WT = MCF-7 cells transfected with wild-type COX-2 gene; and M = MCF-7 cells transfected with Asn^{580}-mutant COX-2 gene. Whole cell lysates were prepared 72 hours after transfection and were subjected to SDS- PAGE followed by western blotting and immunostaining. β-actin served as the "housekeeping" gene.

Figure 4



Effect of COX-2 glycosylation on the migratory potential of MCF-7 cells. Control = MCF-7 cells transfected with empty plasmid; 72/74 kDa = MCF-7 cells transfected with the wild-type COX-2 gene; and 70/72 kDa = MCF-7 cells transfected with the Asn^{580}-mutant COX-2 gene. Seventy-two hours post transfection, cells were subjected to the cell migration assay. Data are averages +/- SD; n = 2 independent experiments.

CONCLUSIONS

- **Although there was no significant change in the migratory potential amongst the three cells groups 72 hours after transfection, E-cadherin expression decreased at both the transcriptional and translational levels in the cells that expressed the Asn^{580}-mutant COX-2 gene for 72 hours.**
- **Transcriptional expression of β-catenin was also reduced 48 and 72 hours post transfection in cells expressing the mutant COX-2 gene.**
- **Since lack of glycosylation at Asn^{580} leads to an accumulation of COX-2 protein within the cell[3], these results indicate that increased levels of COX-2 correlate with decreased expression of both E-cadherin and β-catenin in the MCF-7 breast cancer cells.**

This work is dedicated to the memory of Dr. Millie Hughes-Fulford, former mentor of M.B.S.