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# Cyclooxygenase 2 (COX-2) inhibition neutralized ICAM-1 modulation in liver microenvironment during metastatic development


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## INTRODUCTION

During metastatic progression, the liver microenvironment is modulated to promote tumor development. In liver sinusoids, colorectal cancer cells adhere to liver sinusoidal endothelial cells (LSECs), triggering a pro-metastatic cascade and creating a pro-inflammatory and pro-angiogenic microenvironment. Our group has previously shown that ICAM-1 accounts for the up-regulation of inflammatory molecules such us IL-1β, IL-6, $PGE_2$ and TNF-α within the tumor microenvironment (TME) in early phases of liver colonization and that this process is modulated by the inhibition of COX-2, the limiting enzyme in the synthesis of PGE2. Even though several studies support the role of COX-2 in metastatic progression to the liver, little is known about its role in the angiogenic and fibrogenic response modulated by ICAM-1.

## AIM

Our study aims to analyze the intracellular mechanisms by which ICAM-1 promotes those responses and their modulation by COX-2, focusing on the role of LSECs and hepatic stellate cells (HSCs) crosstalk with metastatic cancer cells.

## METHODS

**First,** we analyzed the effect of tumor activation by soluble ICAM-1 (sICAM-1) and by co-culture with LSECs in COX-2 activity and subsequent production of $PGE_2$ and VEGF, central mediators of the inflammatory and angiogenic responses within the TME.

**Second,** to confirm the involvement of COX-2 activity in the process, we proceeded to inactivate the enzyme by the specific inhibitor Celecoxib. Afterwards, the level of PGE2 and VEGF was quantified by ELISA.

**Finally,** we analyzed the migration of primary mouse LSECs and HSCs, main actors of angiogenesis, and desmoplasic response during liver metastasis, upon activation with Celecoxib treated tumor secretomes after sICAM-1 stimulation.

## RESULTS

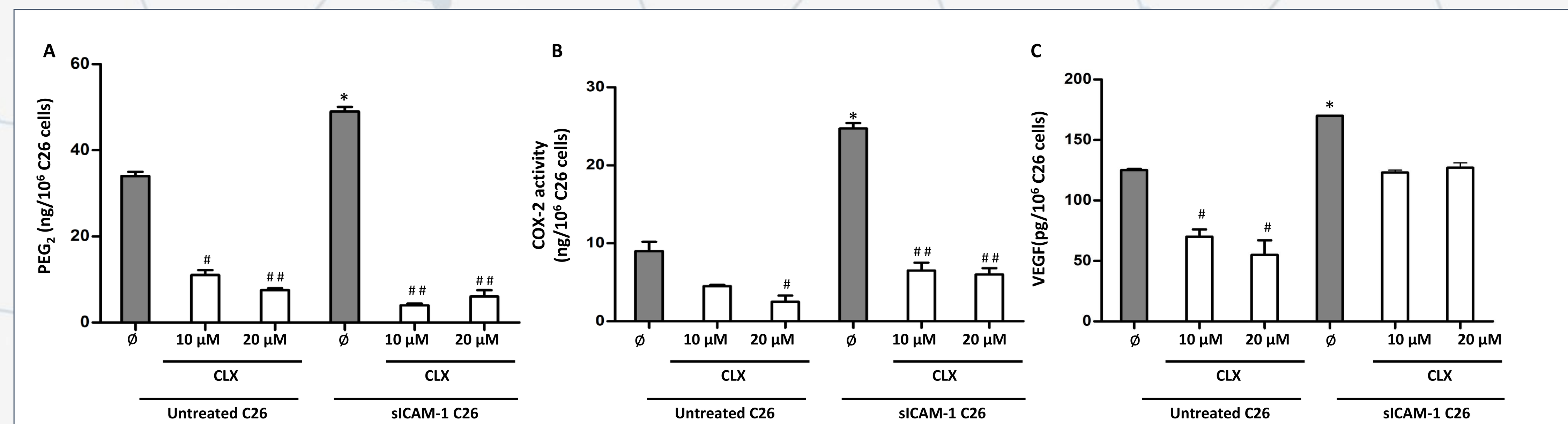



**Figure 1. ICAM-1 induced $PGE_2$ and VEGF production via COX-2 activation on C26 cells.** Inhibition of COX-2 activity with CLX reduced $PGE_2$ and VEGF production after the activation of sICAM-1 in C26 cells. Results were considered significant when p<0'05 (* sICAM-1 treated vs untreated, and # for CLX treated vs CLX untreated) and p<0'01 (##), by "T Student.

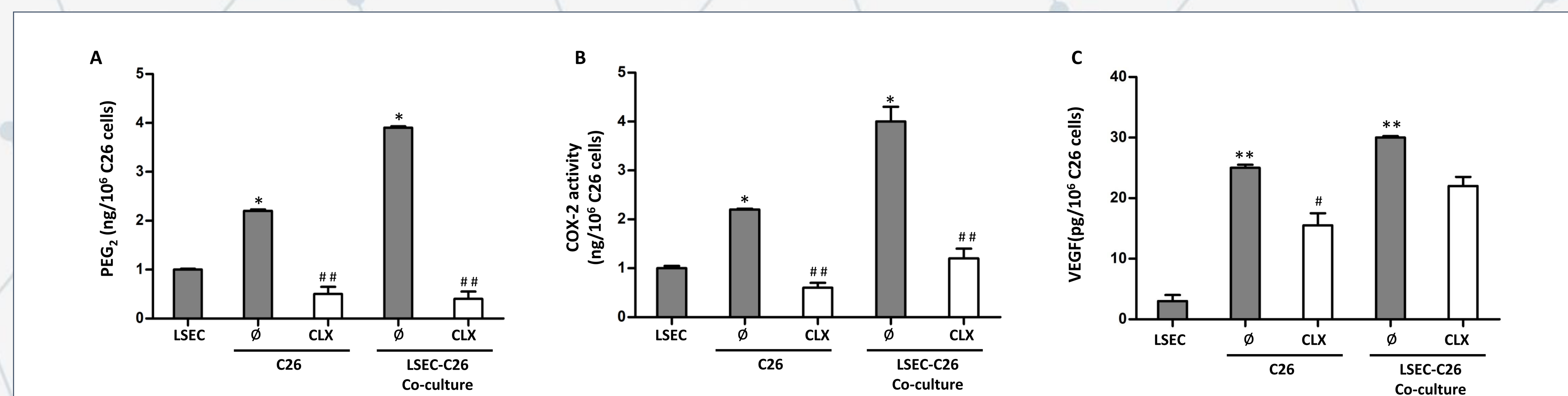



**Figure 2. Effect of COX-2 activity inhibition on $PGE_2$ and VEGF secretion in LSEC-C26 co-cultures.** $PGE_2$ and VEGF production were stimulated by interaction between tumor cells C26 and LSECs. This effect was abrogated after COX-2 inhibition by celecoxib. Results were considered significant when p<0'05 (*, respect control (only LSEC culture) and # for CLX untreated respect CLX treated) and p<0'01 (##), by "T Student".

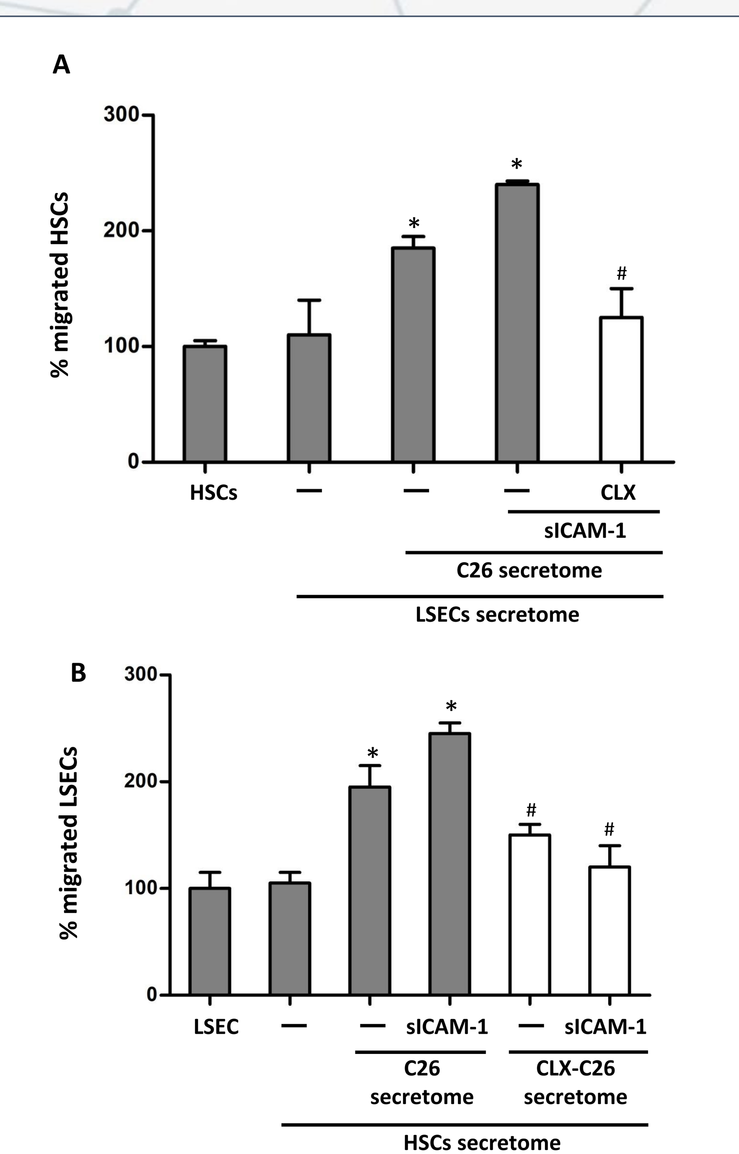



**Figure 3. Reciprocal activation of the migratory activity on stromal cells by ICAM-1 stimulation of tumor cells is dependent on COX-2 activity.** HSCs migratory capacity increased by tumors-activated LSECs secretome. Moreover, the sICAM-1 activation in C26 increased the migratory capacity of HSCs that was reduced by COX-2 inhibition. In LSECs, activated HSCs secretome increased the capacity migrations, especially after sICAM-1 activation of tumor cells. This capacity was reduced by COX-2 inhibition. Results were considered significant when p<0'05 (*. Respect HSCs (A) and LSECs (B) and # for CLX untreated respect CLX treated) and p<0'01 (**), by "T Student".

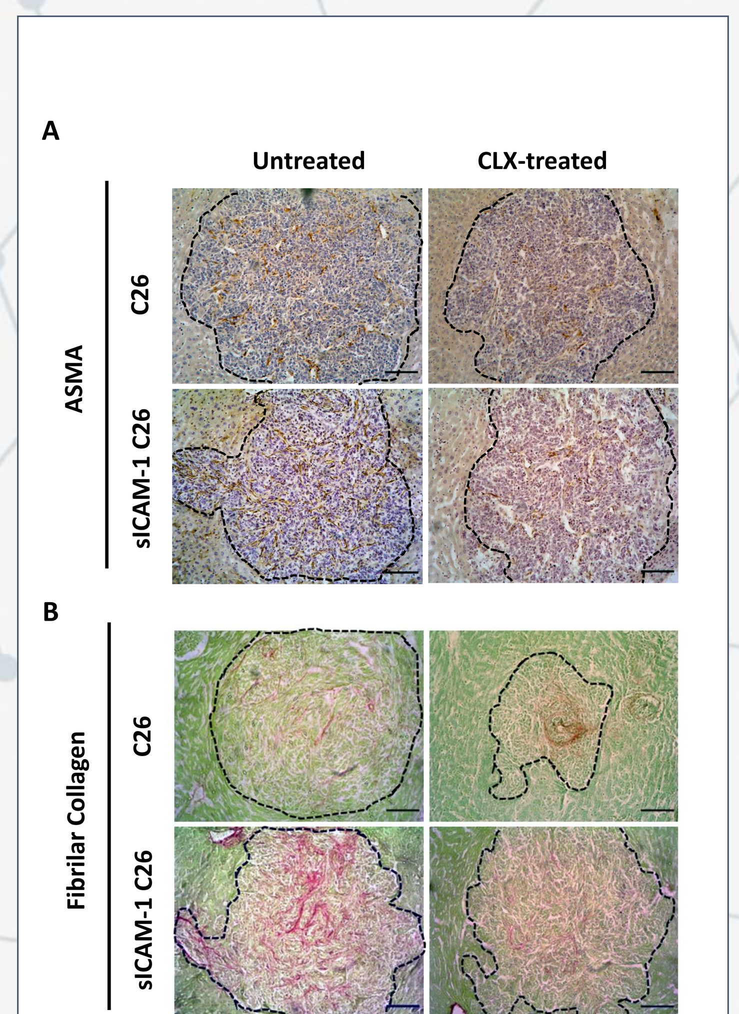
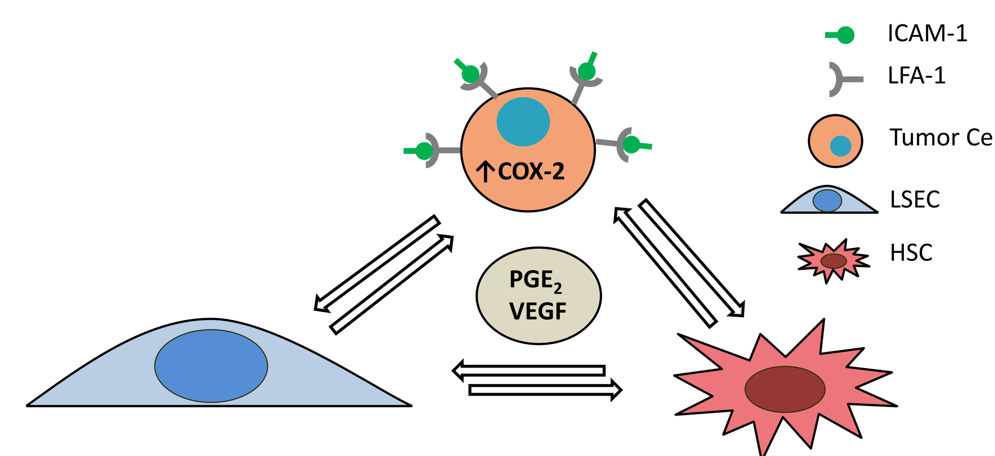



**Figure 4. *In vivo* recruitment of ASMA⁺ cells and collagen deposition in livers of C26-bearing mice.** Liver collected from sICAM-1- activated C26 bearing mice showed increase ASMA⁺ cell recruitment and collagen deposition compared to those liver collected from control C26-bearing mice. This increase was abrogated by treatment with the COX-2 inhibitor celecoxib.

## CONCLUSIONS

Tumor cell activation with sICAM-1 increased intracellular COX-2 activity resulting in an increased $PGE_2$ and VEGF secretion in the supernatants. The same effect was reported in co-cultures of tumor cells and LSECs. COX-2 inhibition by Celecoxib decreased COX-2 activation and the production of both $PGE_2$ and VEGF by tumor cells and co-cultures. Besides, secretomes of sICAM-1 stimulated tumor cells burst the migration of both LSECs and HSCs compared to that of untreated tumor cells throiugh a reciprocal interaction. This sICAM-1 mediated effect was abrogated upon Celecoxib treatment of tumor cells, leading to decreased migration of LSECs and HSCs.

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