

INTRODUCTION:

Protein Convertases are a family of 9 mammalian serine secreted proteases (PCSK1, PCSK2, FURIN, PCSK4, PCSK5, PCSK6, PCSK7, SKI1, PCSK9) that modulate the biological activity of proteins by removing aminoacide chains. They are involved in the activation of a wide variety of secretory proteins that are essential for the maintenance of homeostasis, such as cytokines, proteases, adhesion molecules and growth factors (GF) and GF receptors, among others. Indeed, it is known that proprotein convertase subtilisin/kexin type 9 (PCSK9) increases circulating levels of low-density lipoprotein (LDL) but emerging investigations revealed also novel functions; including cell apoptosis, inflammatory response, neuronal development and tumor metastasis.

Cancer Stem Cells (CSCs) have been recently pointed out to be responsible for cancer initiation, progression, metastasis, recurrence and drug resistance. This is because any cell of a lineage that is not terminally differentiated, and can still proliferate, can potentially give rise to cancer.

Our study aimed to further elucidate the role of PCSK9 in cancer. Previously, our studies demonstrated a different expression of PCSK9 in CSCs and parental cells from metastatic and non-metastatic human colorectal adenocarcinoma cells lines. After using a PSCK9 inhibitor, PF-06446846, we analyze the expression both at the protein and gene expression levels and we studied the role of PCSK9 on the cellular functions of colon CSC. On the other hand, the co-expression of CD133 (CSC marker) and PCSK9, has been studied by immunofluorescence in biopsies of primary and metastatic tumors.

RESULTADOS:

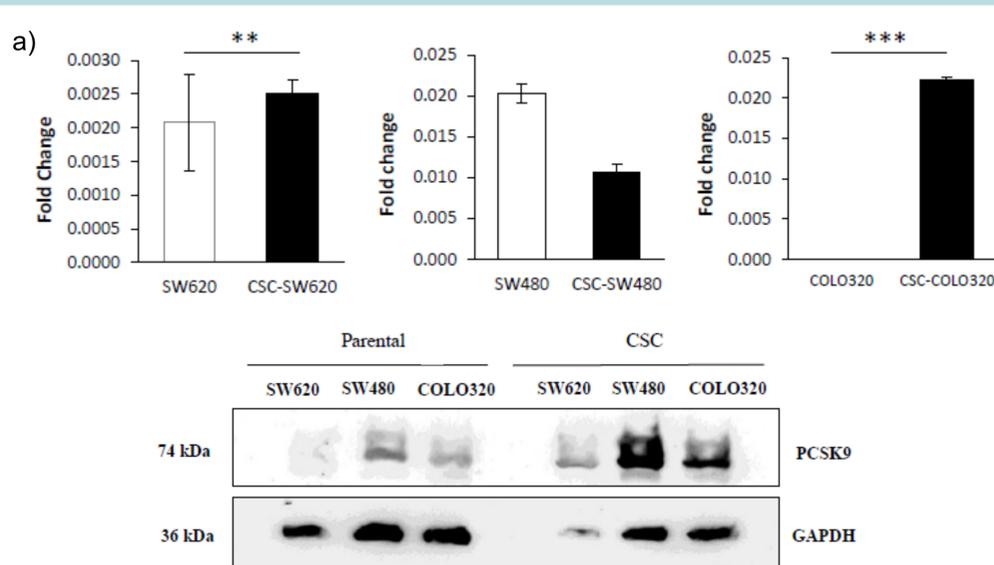


Figure 1: PCSK9 expression was higher in CSCs than in parental cells.

a) PCSK9 expression analyzed by RT-PCR in SW620 and COLO320 cell lines is higher in the CSCs. Amplification results for samples SW620, CSC-SW620, SW480, CSC-SW480, COLO320, and CSC-COLO320 are shown in Fold Change values; in white, parental cells, and in black, CSC. The PCSK9 Ct values for each cell line were subtracted from the mean GAPDH Ct values (ΔCt) and then the fold change value ($2^{-\Delta Ct}$) was calculated. Finally, the mean of these values, standard deviation, standard error and T-Student values were calculated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

b) The results of the Western Blot of proteins extracted from human colon and rectum cell lines are shown, the bands correspond to samples SW620, SW480, COLO320, CSC-SW480, CSC-SW620 and CSC-COLO320, respectively. The results were normalized to the GAPDH control.

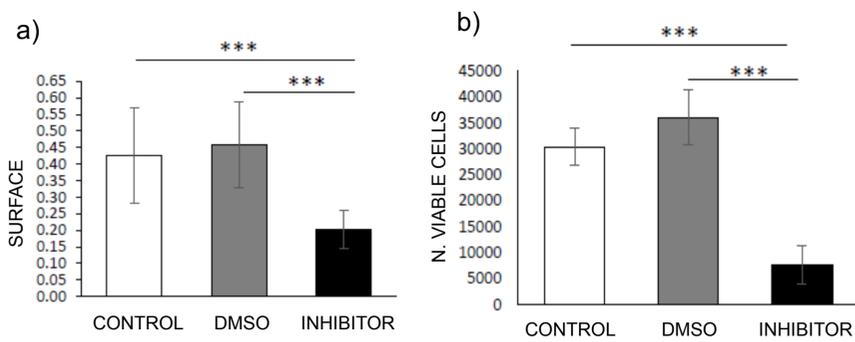


Figure 2: Inhibition of PCSK9 significantly decreased CSC surface area and viability.

a) The surface area of the CSC-SW480 cells was measured 6 days after the addition of the inhibitor. Control with blank, samples with DMSO in gray and samples with DMSO + Inhibitor in black. Five photos of the well were taken in each condition, after which the surface area was calculated.

b) The number of CSC-SW480 cells viability was analyzed by measuring absorbance at 490 nm. Control with white, samples with DMSO in gray, and samples with DMSO + Inhibitor in black.

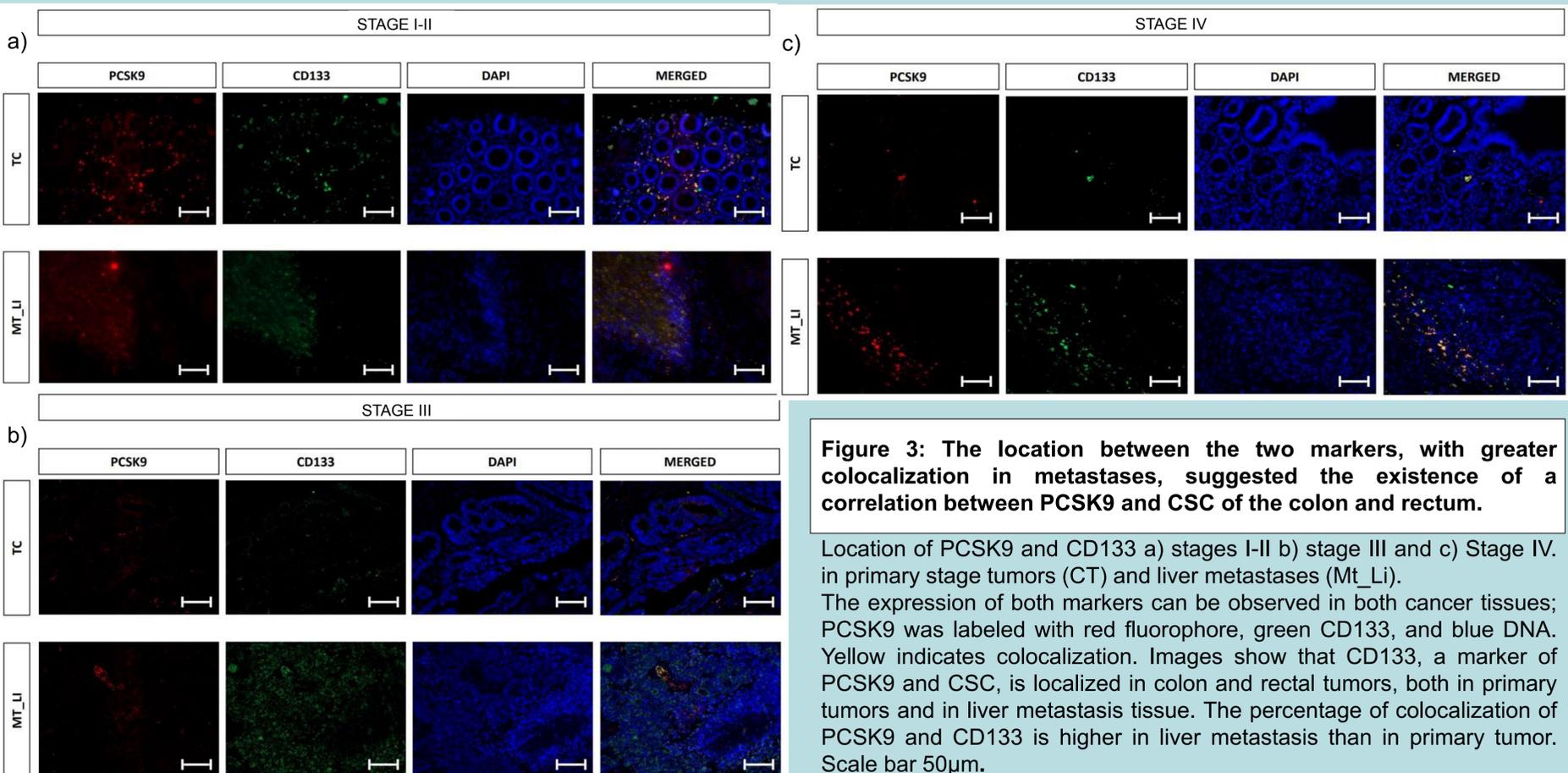


Figure 3: The location between the two markers, with greater colocalization in metastases, suggested the existence of a correlation between PCSK9 and CSC of the colon and rectum.

Location of PCSK9 and CD133 a) stages I-II b) stage III and c) Stage IV. in primary stage tumors (CT) and liver metastases (Mt_Li). The expression of both markers can be observed in both cancer tissues; PCSK9 was labeled with red fluorophore, green CD133, and blue DNA. Yellow indicates colocalization. Images show that CD133, a marker of PCSK9 and CSC, is localized in colon and rectal tumors, both in primary tumors and in liver metastasis tissue. The percentage of colocalization of PCSK9 and CD133 is higher in liver metastasis than in primary tumor. Scale bar 50 μ m.

CONCLUSIONS:

- PCSK9 expression was higher in CSCs than in parental cells.
 - Inhibition of PCSK9 significantly decreased CSC viability
 - The location between the PCSK9 and stem cell marker, with greater colocalization in metastases, suggested the existence of a correlation between PCSK9 and CSC of the colon and rectum.
- PCSK9 may play a role in tumor development and metastasis. His inhibition could be potentially use for future cancer therapy.