Reviewer’s report

Title: FAP-overexpressing fibroblasts produce an extracellular matrix that enhances invasive velocity and directionality of pancreatic cancer cells

Version: 1 Date: 16 February 2011

Reviewer: Yves Boucher

Reviewer’s report:

Lee et al determined the effects of a 3-dimensional matrix produced by fibroblasts expressing the fibroblast activation protein (FAP) on fibronectin organization and pancreatic cancer cell migration. The results show that matrices produced by fibroblasts expressing FAP organize fibronectin fibrils in parallel patterns, whereas FAP-negative fibroblasts or inhibition of FAP activity reduces the parallel orientation of the fibrils. The remodeling of the matrix by FAP-positive fibroblasts was associated with the enhanced directionality and velocity of pancreatic cancer cells. It is also shown that the velocity and migration directionality is dependent on integrins #1 and #5#1. The findings are of interest, but there are several weaknesses in the description of the experimental model, interpretation of results and additional experiments should be performed to provide greater depth.

Major Compusory Revisions

The authors propose that the remodeling of fibronectin fibrils by fibroblasts affects cancer cell migration. #1-integrin or alpha #5#1 inhibition reduces cancer cell migration, however the authors did not determine if the changes in migration induced by integrin inhibition are associated with modifications in matrix remodeling. Furthermore, fibronectin is not a FAP substrate, so why does the inhibition of FAP activity affect fibronectin remodeling? Do FAP-positive fibroblasts also affect the remodeling of collagen I fibers?

Why were the effects of matrices produced by FAP-, FAP+, or FAP+inhibitor fibroblasts or the inhibition of #1 integrin or alpha #5#1 only studied with only one pancreatic cancer cell line. To determine the significance of the results other cell lines should also be tested.

The upregulation of FAP in fibroblasts increases their expression of collagen I, fibronectin and #SMA and treatment of FAP+ matrices with a FAP inhibitor further increases the expression of fibronectin and #SMA. It is unclear if the increase in fibronectin and #SMA expression is significantly higher between matrices treated with or without the FAP inhibitor.

Also, the treatment of experimental tumors with a FAP inhibitor increases the intratumoral collagen content and decreases the number of myofibroblasts (#SMA-positive cells) (Santos et al., 2009, J. Clin. Invest., 119: 3613). In contrast, in the present study the inhibition of FAP activity does not affect the
expression of collagen I and increases the #SMA-positive expression. These differences in FAP activity between in vitro and in vivo results should be acknowledged and explained.

For the migration studies the cancer cells were plated on the matrix formed by the fibroblasts. However, it is not specified if cancer cell migration was measured and reported for cells on top or inside the gel.

How many experiments were performed to determine the average values of migration velocity and directionality?

Discretionary Revisions

What is the composition of the matrix produced by the fibroblasts, is there a significant accumulation of collagen type I fibers in the matrix?

The immunostaining results suggest that FAP is expressed by most fibroblasts in pancreatic cancer tissue sections. In a more detailed study it was found in pancreatic cancer sections that the expression of FAP by fibroblasts decreases has a function of the distance from tumor cells (Cohen et al., 2008, Pancreas 37: 154). The expression of FAP in pancreatic cancer tissue presented in figure 1 is not really necessary and should be removed.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.