Myoferlin, a key regulator of VEGF secretion in human pancreatic cancer

Fahmy Karim*, Gonzalez Arnaud*, Francart Marie-Emilie, Turtoi Andrei, Thiry Marc, Castronovo Vincent, Peulen Olivier

*Both authors contributed equaly to this work

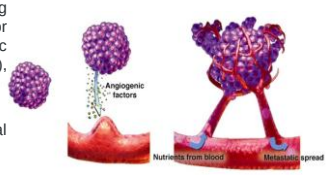
GIGA-Cancer, Laboratory of Metastasis Research, University of Liège

Introduction

Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression. Its inhibition may be a valuable new approach to cancer therapy. Avascular tumors are severely restricted in their growth potential and appearing as a whitish pale mass because of their lack of blood supply. Reports has proved that the main route of metastasis is blood circulation. For tumors to develop in size and metastasize, they must make an "angiogenic switch" through a perturbation of the local balance of proangiogenic versus antiangiogenic factors. Frequently, tumors overexpress proangiogenic factors, such as vascular endothelial growth factor (VEGF), allowing them to make this angiogenic switch.

Our laboratory has identified myoferlin, a member of the ferlin protein family, as a new accessible biomarker for human pancreatic ductal adenocarcinoma. Ferlin protein family has been reported to participate in plasma membrane fusion, repair, and protein trafficking.

The current work in our laboratory reports myoferlin as a key regulator in VEGF secretion in pancreatic ductal adenocarcinoma by controlling the exocytosis of VEGF secretory granules in the tumor stroma. Further investigation are on going to reveal its mechanism of action.



Results

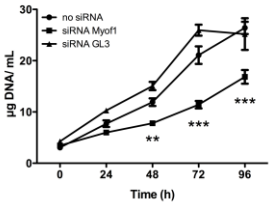


Figure 1: Proliferation test/DNA quantification test showing the effect of myoferlin silencing on the proliferation of BxPC-3 cells *in vitro*. Results show a decrease in proliferation by a decrease in DNA quantity after myoferlin silencing. (2-way ANOVA, **P<0,01, ***P<0,001)

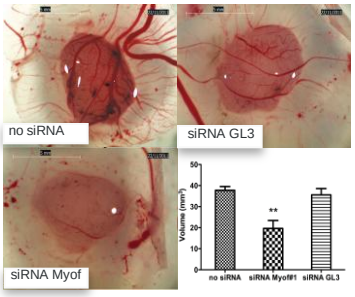
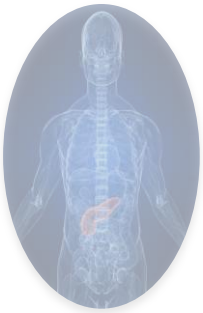


Figure 2: CAM assay/*In vivo* proliferation test showing the effect of myoferlin silencing on the proliferation and angiogenesis of BxPC-3 cells grown on CAM. a) CAM assay results showing a whitish, pale and smaller tumor size after myoferlin silencing. b) Tumor volume quantification showing a significant reduction of the tumor volume in the myoferlin silencing condition. (1-way ANOVA, **P<0,01)



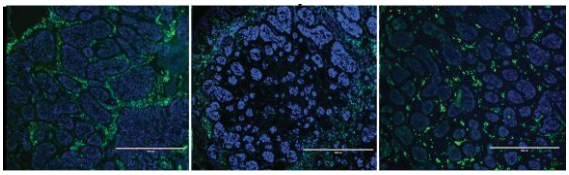


Figure 3: SNA staining showing the effect of myoferlin silencing on the vascularization of the tumor mass in a CAM assay. Results show a decrease in vascularization on the myoferlin silencing condition. (Blue= nuclei, Green= blood vessels)

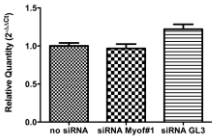


Figure 4: PCR results showing effect of myoferlin silencing on VEGF transcription. Results show that myoferlin silencing does not affect VEGF transcription

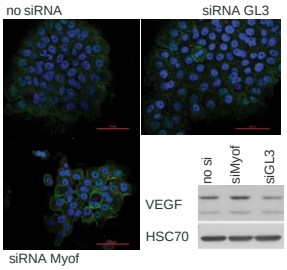


Figure 5: Immunofluorescence and immunoblotting techniques showing the effect of myoferlin silencing on VEGF secretion of BxPC-3 cells. Immunofluorescence results show a cytosolic VEGF accumulation in myoferlin silencing condition. Western blot results confirms the increase of VEGF in the total cell extract after myoferlin silencing

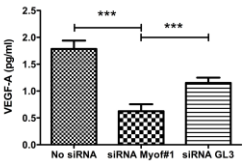


Figure 6: ELISA assay showing the effect of myoferlin silencing on VEGF secretion of BxPC-3 cells in conditioned medium. Results show that myoferlin silencing decreases VEGF secretion by BxPC-3 cells in their conditioned medium. (1-way ANOVA, ***P<0,001)

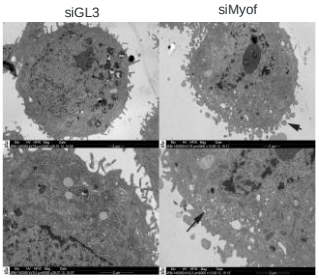


Figure 7: Electron microscopy technique showing the effect of myoferlin silencing on ultrastructure. Results show accumulation of susceptible VEGF secretory granules in the myoferlin silencing condition

Summary

Myoferlin identification as a biomarker of pancreatic ductal adenocarcinoma implies an important role of myoferlin in the cancer progression and metastasis.

We showed that myoferlin silencing reduces the proliferation of BxPC-3 cell by 50% both *in vitro* and *in vivo*. *The reduction of the tumor mass grown on CAM was accompanied by a decrease in vascularization implying a reduction in angiogenesis. This observation was confirmed by the whitish pale appearance of the tumor mass as well as by SNA staining. Further investigations showed that myoferlin does not seem to affect VEGF transcription as seen in the PCR results. However, it has been observed a retention of the VEGF at the peri-plasmalemmal's area by immunofluorescence staining and western blotting . ELISA assay confirmed the VEGF concentration decrease in the conditioned medium of BxPC-3 cells in spite of fact the VEGF transcription is not altered by myoferlin silencing. Finally, preliminary electron microscopy analysis showed the retention of secretory vesicles in the peri-plasmalemmal's area believed to contain VEGF.*

Literature emphasized the implication of certain ferlin protein family in membrane fusion and as well as in endocytosis and in exocytosis. Recently, groups reported the implication of otoferlin, an homolog of myoferlin, in secretory granules exocytosis in the cochlear hair cells in the ears. This results strengthen our hypothesis in the implication of myoferlin in the exocytosis of VEGF granules in BxPC-3 cells.

Contact : karim.fahmy@student.ulg.ac.be