Myoferlin: an indispensable component in VEGFA secretion by pancreas cancer cells

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Introduction

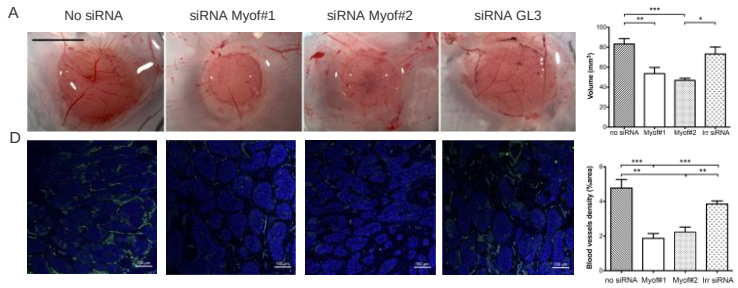
Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression. Its inhibition may be a valuable new approach to cancer therapy. Avascular tumors are severely restricted in their growth potential and appear as a whitish pale mass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

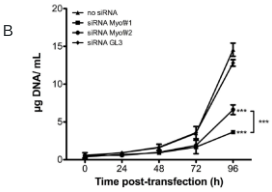
Pancreatic ductal adenocarcinoma is one of the most deadly forms of cancers with no satisfactory treatment to date. Recent studies have identified myoferlin, a ferlin family member, to be overexpressed in human pancreas adenocarcinoma where its expression was associated to bad prognosis. However, the function of myoferlin in pancreas adenocarcinoma has not been reported. In other cell types, myoferlin is involved in several key plasma membrane processes such as fusion, repair, endocytosis and tyrosine kinase receptor activity.

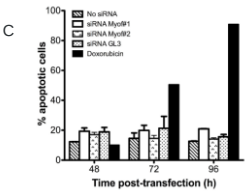
The current work reports myoferlin as a key regulator in VEGF-A secretion in pancreatic ductal adenocarcinoma by controlling the exocytosis of VEGF-A secretory granules in the tumor stroma.

Results

Myoferlin silencing reduces pancreas cancer cells growth *in vivo/in vitro* and reduces angiogenesis



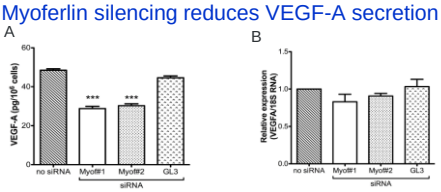




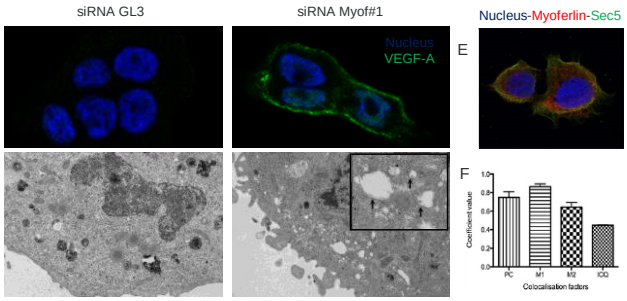
(A) Myoferlin silencing reduces *in vivo* BxPC-3 tumor grown on CAM by 50% and (B) reduces *in vitro* cell proliferation by 80% (C) without induction of apoptosis.

(D) In vivo, myoferlin silenced BxPC-3 tumor grown on CAM show a decrease in blood vessels density inside the tumor core as seen the *Sanbucous nigra* staining.

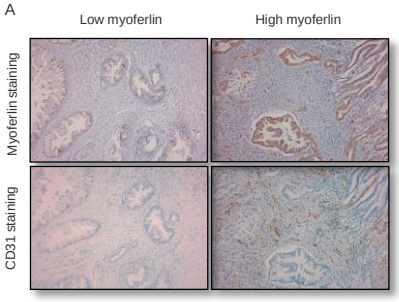
Myoferlin silencing reduces VEGF-A secretion



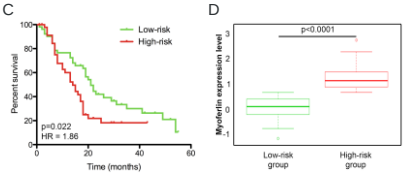
(A) Myoferlin silencing in BxPC-3 cells provokes a decrease in VEGF-A concentration in the conditioned medium (B) without alteration of the gene transcription. (C) Immunofluorescence shows a cytosolic accumulation of VEGF-A in myoferlin silenced condition. (D) Electron microcopy reveals the accumulation of vesicle-like structures in the vicinity of the plasma membrane unable to fuse with the plasma membrane and release their cargo, suspected to be VEGF-A. (E) Immunofluorescence proposes the colocalization of myoferlin with sec5/Exoc2, a component of a complex essential for targeting exocytic vesicles, mainly at the periphery of the cells. (F) Colocalization of myoferlin and Sec5/Exoc2 was evaluated by 4 different correlation parameters :(PC: Person's Coefficient; M1 and M2 : Mander's Coefficient; ICQ: Li's Intensity Correlation Quotient).



Myoferlin staining extent associates with blood vessel density and survival in PDAC



(A) PDAC and non cancerous pancreas sections staining for myoferlin and CD31. (B) Results show that the CD31 staining isn't different between low and high moyferlin staining of non cancerous panreas. However, a significant difference is seen in PDAC sections: high myoferlin staining cases have high CD31/HPF and low myoferlin staining have low CD31/HPF.



(C) Kaplan–Meier curve of a publically available dataset (Pancreas Expression Database) allowed us to classify PDAC patients into two groups significantly different in terms of survival; (D) where myoferlin expression level is aslo significantly higher in high risk group.

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 reduced the proliferation of BxPC-3 cell by 50% in vivo and 80% in vitro without an increase in apoptosis. 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 VEGF-A concentration decreases in the conditioned medium of BxPC-3 myoferlin silenced cells although myoferlin silencing doesn't affect VEGF-A transcription as seen in the PCR results. However, it has been observed a retention of the VEGF-A at the peri-plasmalemmal's area by Immunofluorescence staining. Also, electron microscopy analysis showed the retention of vesicles-like structure in the peri-plasmalemmal's area believed to contain VEGF-A. Finally, Immunofluorecence also show that myoferlin partially colocalizes with sec5, a component of a complex essential for targeting exocytic vesicles, strengthen the hypothesis of a role in exocytosis. *In PDAC patients sections, immunoperoxidase staining of both myoferlin and CD31 showed that myoferlin staining extent is associated to blood vessels density. Finally dataset analysis revealed that myoferlin expression is associated to patients' survival.*

"Myoferlin plays a key role in VEGFA secretion and impacts tumor-associated angiogenesis in human pancreas cancer"
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