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Introduction

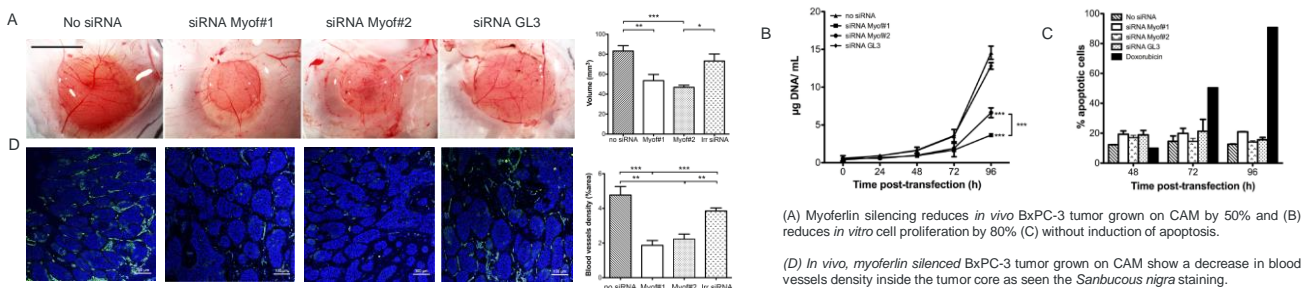
Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression. Its inhibition may be a valuable new approach to cancer therapy. Avascular tumors are severely restricted in their growth potential and appear as a whitish pale mass 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.

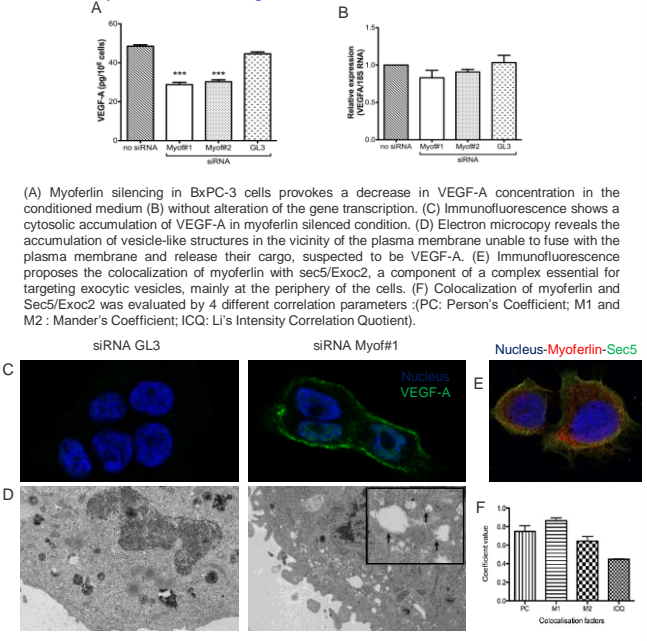
The current work reports myoferlin as a key regulator in VEGF-A secretion in pancreatic ductal adenocarcinoma by controlling the exocytosis of VEGF-A secretory granules in the tumor stroma.

Results

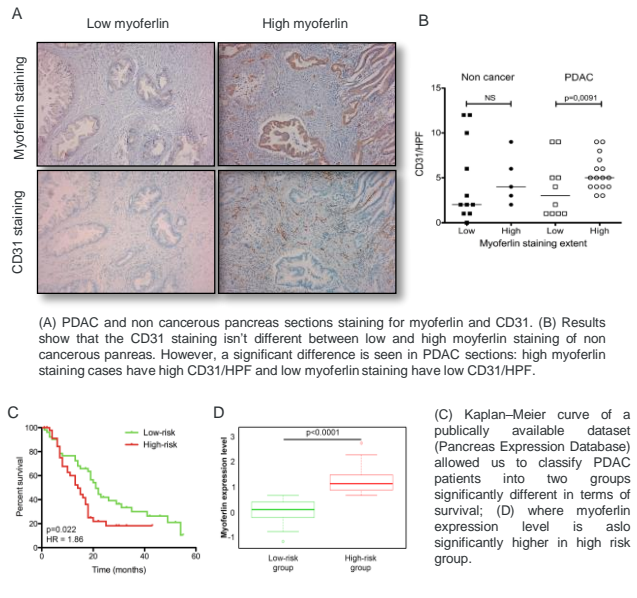
Myoferlin silencing reduces pancreas cancer cells growth *in vivo/in vitro* and reduces angiogenesis



Myoferlin silencing reduces VEGF-A secretion



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



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, immunofluorescence 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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