Investigating the Role of Melanoma-secreted FSTL1 in Cancer Angiogenesis

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Abstract

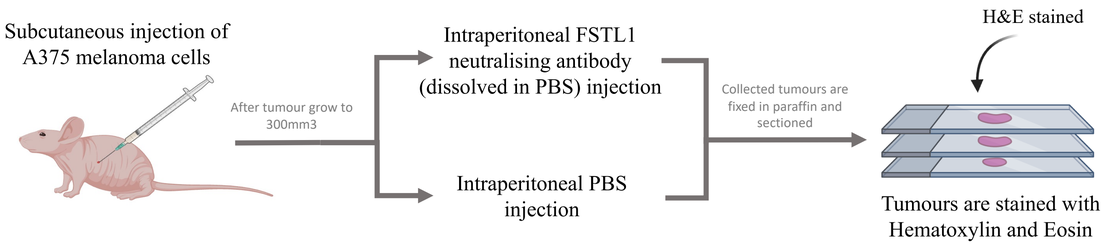
Melanoma is the deadliest skin cancer with high tendency to undergo metastasis and develop drug resistance. FSTL1 expression is induced by melanoma oncogenes, yet its effect on melanoma remains unknown. To test the hypothesis that melanoma-secreted FSTL1 promotes angiogenesis in vivo, we subcutaneously injected A375 human melanoma cells into nude mice. We then performed histological analysis of the treated and untreated tumour tissue. We found that injection of FSTL1-neutralizing antibodies caused a reduction in tumour growth rate and smaller blood vessel size in the final tumour. To further explore the effect of FSTL1 in the tumour microenvironment, we performed melanoma assembloid coculture experiments to mimic the complete tumour microenvironment in vitro. Immunohistological analysis of the assembloid model showed that that overexpression of FSTL1 is correlated with more extensive blood vessel colonisation as compared to the control group, while treatment with the neutralising antibody attenuated these effects. Altogether, the results show that FSTL1 promotes cancer angiogenesis in melanoma.

Introduction

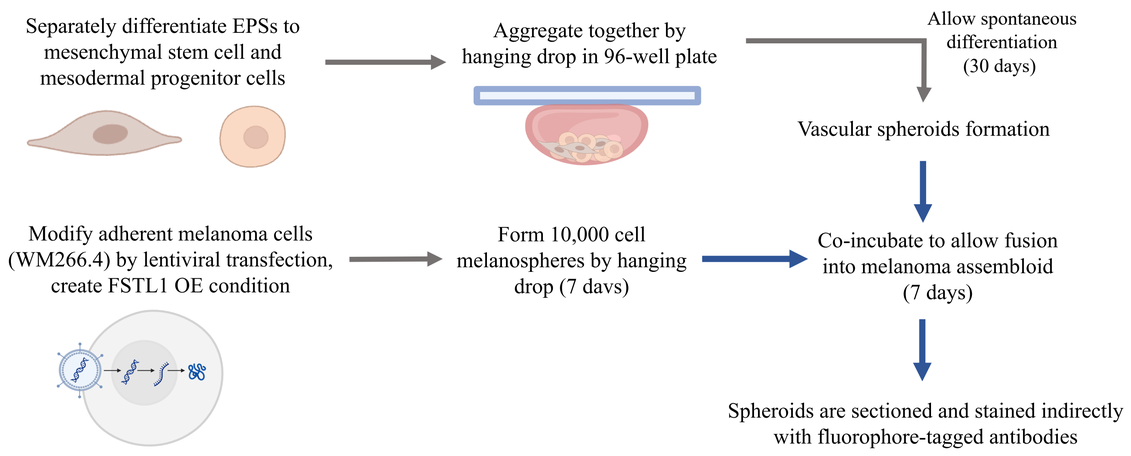
FSTL1 (Follistatin-like 1) is a protein with many roles in human physiology and cancer. The roles of FSTL1 in different cancer types are very diverse. While FSTL1 is linked to malignancy and metastasis in hepatocellular carcinomas (HCC), it inhibit metastasis in lung cancer. The purpose of this study is to understand the role of FSTL1 in melanoma angiogenesis, which is necessary to supply nutrients and oxygen, and to undergo metastasis. Studying the role of FSTL1 in melanoma will allow us to assess its feasibility as a candidate for drug treatment.

Methodology

H&E staining



Immunofluorescence (IF)



"Images are created with BioRender.com.

Results

Experiment 1 : FSTL1 neutralising antibody reduces tumor growth and blood vessels area

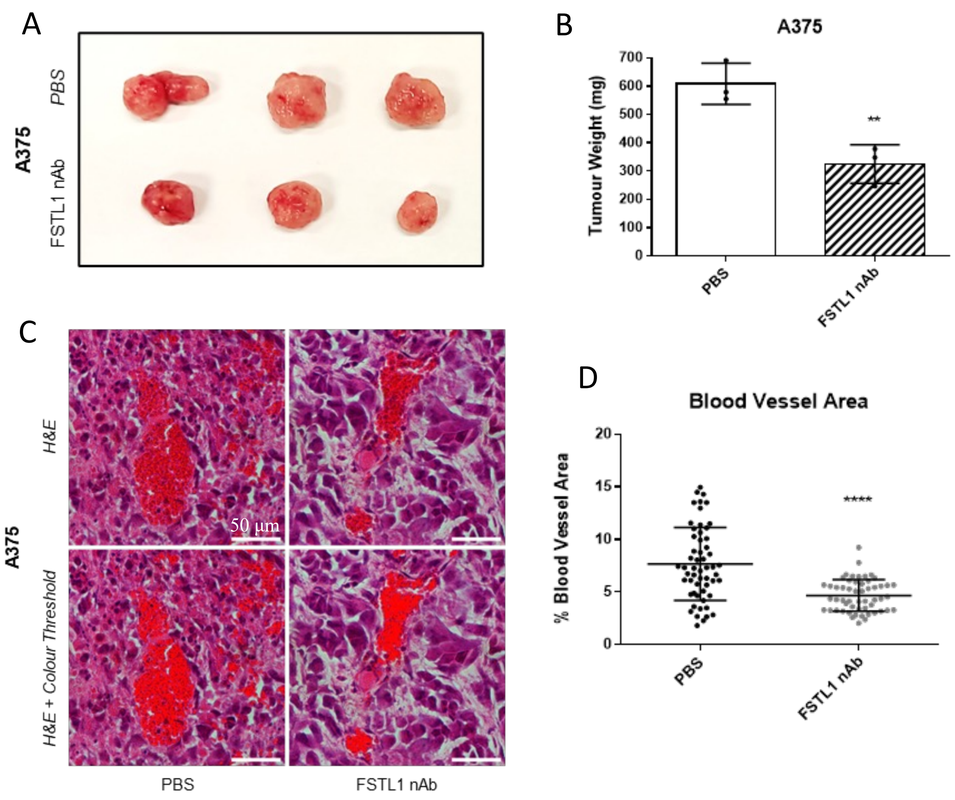


FIG 1 (**A**) The size of tumours extracted from mice with or without FSTL1 nAb added. (**B**) The weight of tumour with or without FSTL1 nAb added, p <0.01. (**C**) H&E staining showing the blood vessel area of the collected tumor samples. The microvessels were imaged at 40x and blood vessel area was measured by colour thresholding. 60 images were taken from 3 sections from 2 different tumours collected. (**D**) Quantification of the blood vessel area. p< 0.0001. PBS = phosphate buffer saline. nAb = neutralizing antibodies. H&E = Hematoxylin and Eosin

Results

Experiment 2 : FSTL1 overexpression promotes blood vessel colonisation in melanoma assembloids

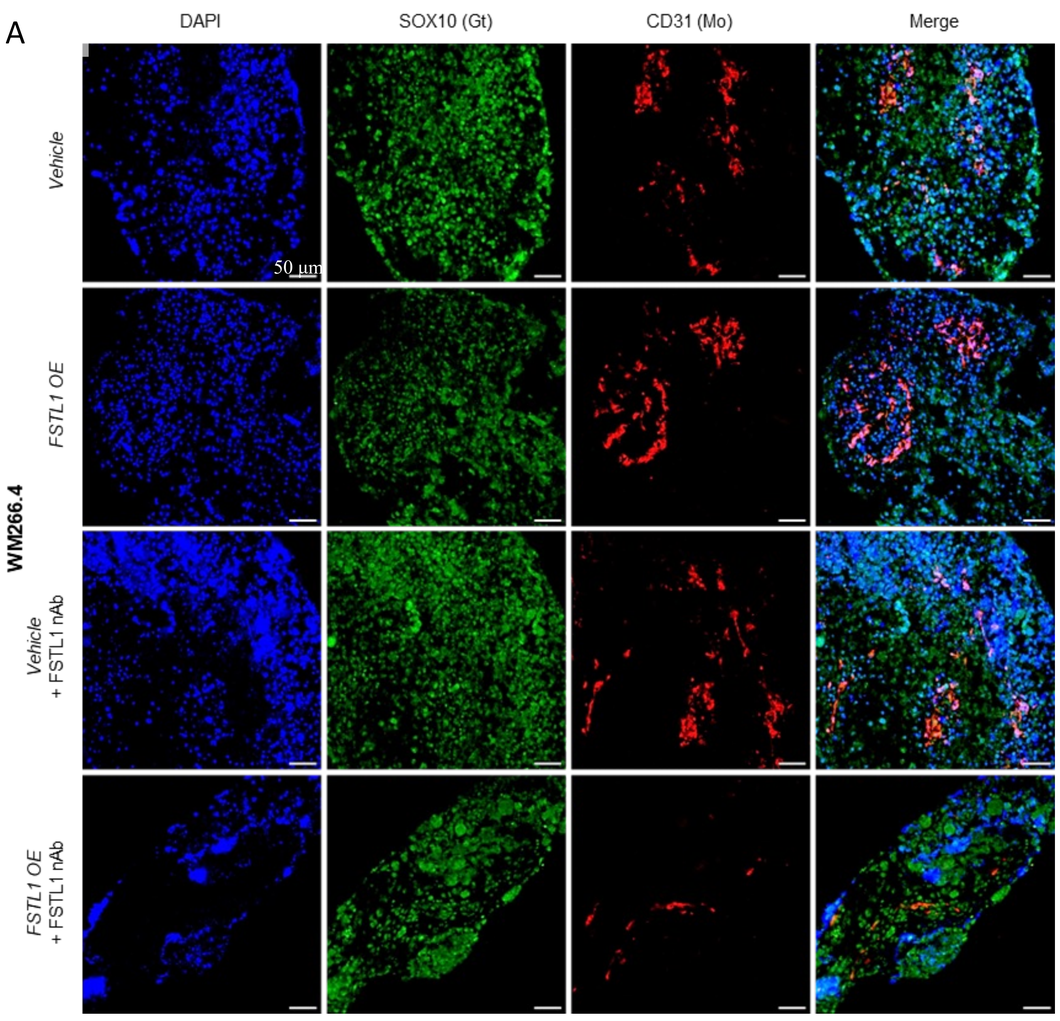


FIG 2 (**A**) Immunofluorescent images of spheroids imaged at 20x. The spheroids are stained with DAPI (blue), SOX10 (green), CD31 (red). The fourth column shows the merged image of the 3 markers. The organization of blood vessels in tumours are visualized. (**B**) Quantification of the area stained with CD31 marker indicates the proportion of blood vessel area in spheroids. DAPI = diaminophenylindoles, stains DNA . SOX10 (Gt) = SRY-Box Transcription Factor 10 (Goat-anti-human), stains melanoma cells. CD31 (Mo) = cluster of differentiation 31 = Platelet endothelial cell adhesion molecule (Mouse-anti-human), stains blood vessels

Discussion

Experiment 1

- Compared with the control, injection of FSTL1 nAb is correlated with smaller tumour size and lighter tumour weight (Figure 1A, B).
- At cellular level, the addition of FSTL1 nAb reduce the percentage of blood vessel area in tumour (Figure 1C,D).

➢ FSTL1 nAb hinders in vivo tumour growth and angiogenesis by suppressing FSTL1 functioning.

Experiment 2

- CD31 is a marker for observation and quantification of blood vessel area.
- Compared with the control, FSTL1 overexpression increases blood vessel colonisation. (Figure 2A,B)
- But when FSTL1 nAb is added to the FSTL1 OE sample for co-incubation, percentage of CD31-stained area shows no significant difference with control.

➢ FSTL1 overexpression increases the extent of angiogenesis and the addition of FSTL1 nAb completely offsets the effect.

Conclusion

➢ Altogether, our results support the hypothesis that melanoma-secreted FSTL1 promotes angiogenesis in vivo.
➢ To further elucidate the mechanism by which FSTL1 promotes angiogenesis, future efforts are on studying the signalling pathway FSTL1 stimulates through RNA sequencing and phospho-proteomics.

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