Extracellular protease ADAMTS1 plays a relevant role in tumor microenvironment remodeling: stemness and endothelial plasticity in human uveal melanoma

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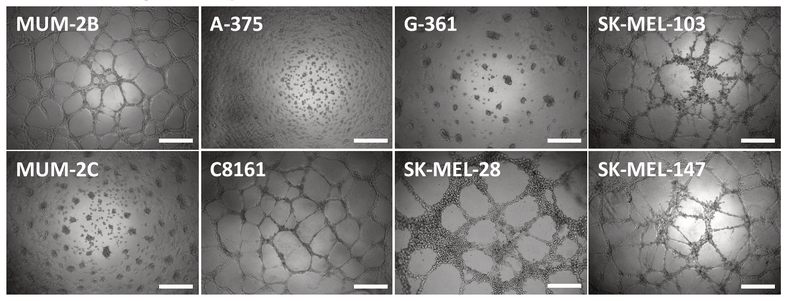
Tumor microenvironment composition and remodeling by extracellular matrix (ECM) proteases have been remarked as key players during tumor growth. Particularly, extracellular protease ADAMTS1 has been associated with tumor development by inducing stemness features and endothelial-like (EL) phenotypes in tumor cells. This connects it to vasculogenic mimicry (VM), a neovascularization mechanism where tumor cells revert to a stem-like state, favoring the acquisition of an EL phenotype. We add here new insights about the contribution of ADAMTS1 and other ECM regulatory molecules to VM, focusing on human uveal melanoma (UVM), a rare but very aggressive cancer. We demonstrated an in vitro EL phenotype for various melanoma cell lines that correlated with ADAMTS1 expression; and that ADAMTS1 inhibition affected in vitro EL attributes and, more importantly, caused a major halt of tumor progression in mice. Stemness features were also compromised in tumor cells, emphasized by our melanoma sphere assays. Finally, taking advantage of expression and clinical data of UVM in TCGA, we disclosed new prognosis elements that sustained our experimental data. To our knowledge, this is the first study reporting the activity of an extracellular protease on the progression of UVM by the induction of stemness and endothelial-like features, and opens the development of new strategies to fight this fatal malignancy.

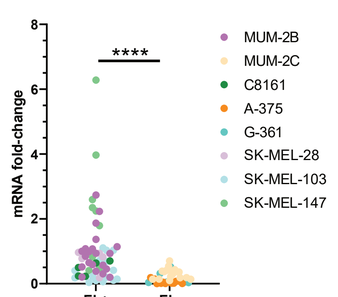
1. ADAMTS1 expression correlates with an EL phenotype

3D Matrigel assay allowed the classification of human melanoma cell lines in EL+ (MUM-2B, C8161, SK-MEL-28, SK-MEL-103 and SK-MEL-147) and EL- (MUM-2C, A-375 and G-361) **(A)**; and qPCR analyses showed that *ADAMTS1* was significantly overexpressed in EL+ cell lines **(B)**.

A. 3D Matrigel assay



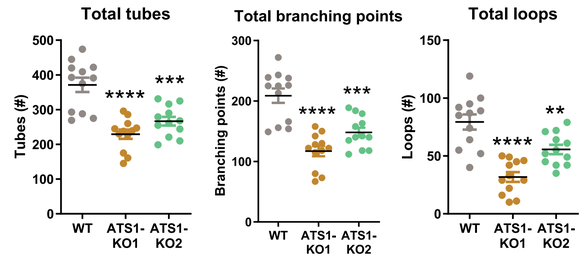
B. ADAMTS1 gene expression



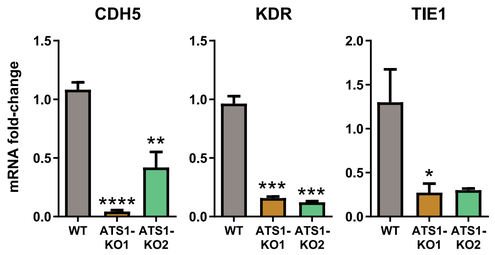
2. ADAMTS1 inhibition affects *in vitro* EL phenotype and gene signature

ADAMTS1 inhibition with CRISPR-Cas9 in MUM-2B resulted in two stable clones (ATS1-KO1 and ATS1-KO2). Unbiased analysis of Matrigel assays with Wimasis software showed an impaired EL phenotype of ATS1-KO cells **(C)**. This inhibition also compromised the EL gene signature, causing a significant downregulation of several endothelial genes related to VM **(D)**.

C. Wimasis analysis of 3D Matrigel assays



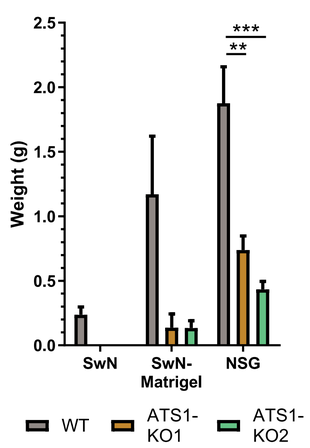
D. EL genes expression



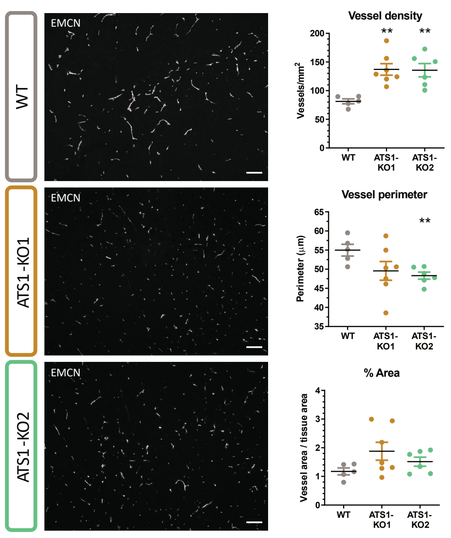
3. ADAMTS1 inhibition affects *in vivo* tumor progression and vasculature

Xenograft studies were executed in different mouse models: Swiss Nude (SwN), SwN using Matrigel in the injection (SwN-Matrigel) and NOD *scid* gamma (NSG). All of them confirmed the relevance of ADAMTS1 for tumor development, as it inhibition blocked tumor growth **(E)**. Analysis of NSG revealed that ADAMTS1 inhibition affected tumor vasculature **(F)** and tumor expression of stemness and endothelial genes **(G)**. IHC analysis revealed a significant reduction of CDH5 and NANOG in ATS1-KO tumors, as occurred with the PAS+ staining, classicaly related with VM **(H)**.

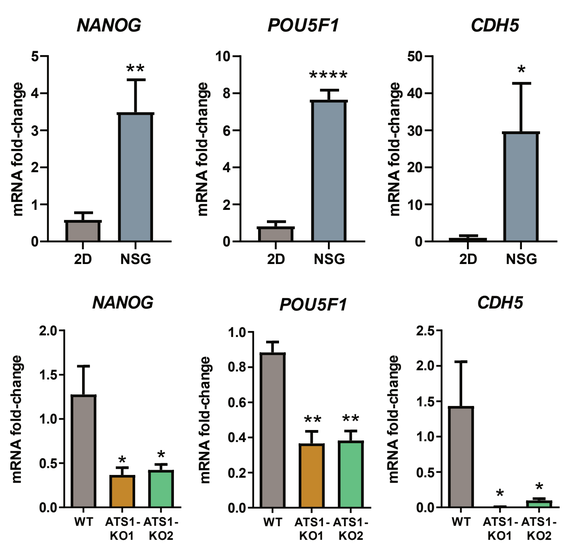
E. Tumor weight



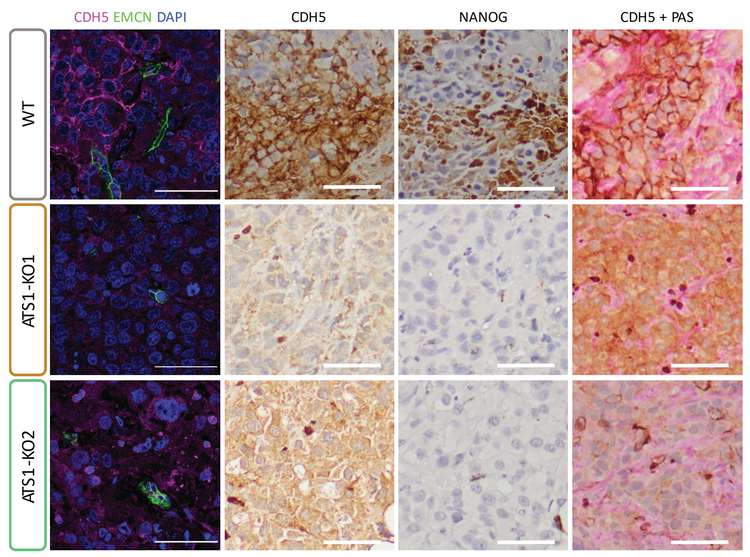
F. Vasculature quantification



G. Stemness and endothelial genes expression



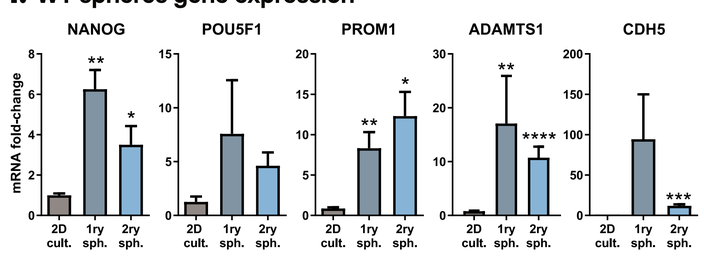
H. IHC analysis of NSG xenografts



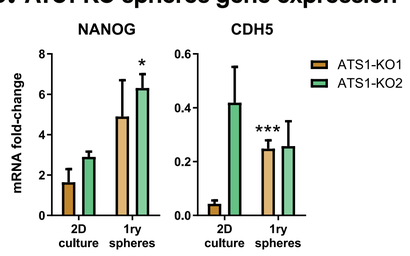
4. ADAMTS1 inhibition compromises *in vitro* stemness capacities

Melanoma sphere (primary and secondary) formation assays with MUM-2B cells showed an enrichment in stemness markers, *ADAMTS1* and *CDH5* in WT spheres **(I)**; that could not be observed in ATS1-KO ones **(J)**. In fact, ATS1-KO cells were completely unable to form melanoma spheres **(K)**, and only the presence of recombinant human ADAMTS1 (rhATS1) in the medium recovered this capacity **(L and M)**.

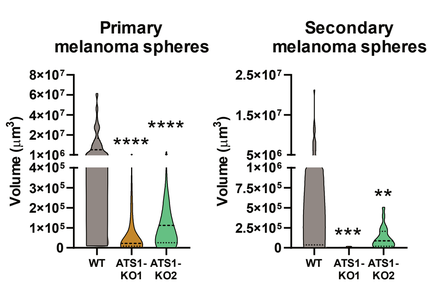
I. WT spheres gene expression



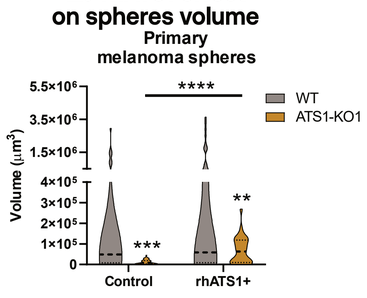
J. ATS1-KO spheres gene expression



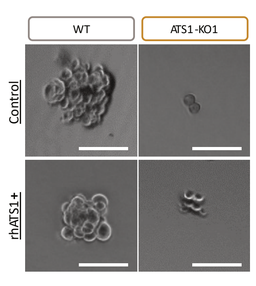
K. Melanoma spheres volume



L. rhATS1 effect on spheres volume



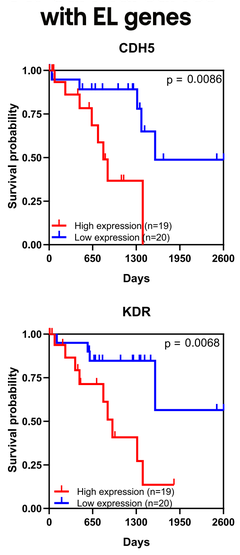
M. Representative spheres



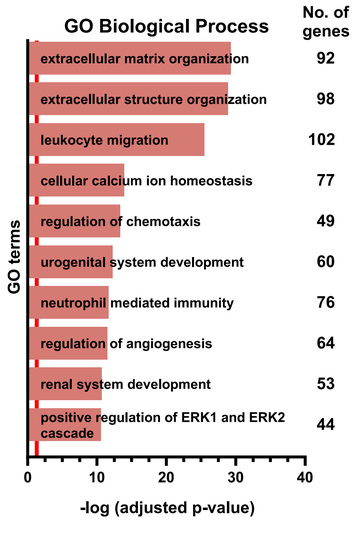
5. Relevance of EL plasticity and ECM regulation in human UVM

Data from TCGA-UVM Project identified *CDH5* and *KDR* as significant poor prognosis factors **(N)**. GO enrichment analysis of positively correlated genes with *CDH5* pointed key features as ECM organization **(Ñ)**, and involved ADAMTSs (*ADAMTS4*, *ADAMTS9*, etc.) which also appeared as poor prognosis factors **(O)**. TCGA-UVM data showed that *ADAMTS1* expression is significantly higher in early stages of UVM **(P)**, which could link it with our experimental results showing its effect on stemness and endothelial plasticity.

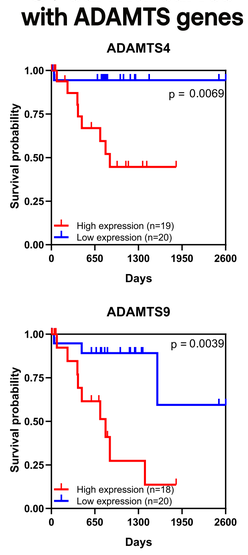
N. Survival curves with EL genes



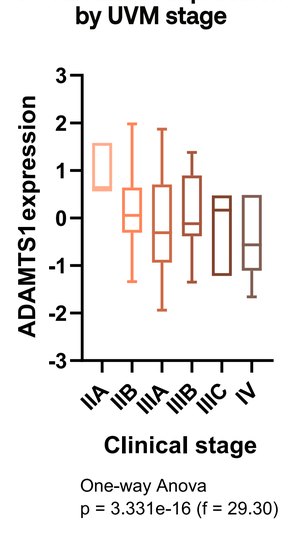
Ñ. GO enrichment analysis



O. Survival curves with ADAMTS genes



P. ADAMTS1 expression by UVM stage



One-way Anova
p = 3.331e-16 (f = 29.30)