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Identification of PAM as a regulator of tissue damage mediated by F1-type Rheumatoid Arthritis Synovial Fibroblasts

Kevin J. Sheridan[1], Emma R. Dorris[1], Christopher D. Buckley[2] and Anthony G. Wilson[1]

[1]University College Dublin Centre for Arthritis Research, Conway Institute, University College Dublin
[2]Rheumatology Research Group, Institute for Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK.

Abstract

Introduction

The single nucleotide variant (SNV) rs26232 is located within the first intron of the *Macrophage Immunometabolism regulator* (*MACIR)* (formerly *C5orf30*), the C allele is associated with the susceptibility to rheumatoid arthritis (RA), and with more severe radiological joint damage. However, rs26232 genotype is not association with either total *MACIR* mRNA or with levels of individual transcript variants suggesting that the genetic association primarily related to another nearby gene.

Aims/Background

Our aims are to investigate the roles of *PAM* in RASF-mediated joint damage.

Methods

Quantitative PCR was used to measure gene expression in RASFs. Gene knock-down was achieved using siRNA technology. Matrigel-coated Boyden transwell chambers were used to measure RASF invasion and migration was assayed using the scratch assay. Proliferation was quantified using BrdU ELISA and Caspase 3/7 levels were used to measure apoptotic activities.

Results

Online databases (GTEx portal and Open Targets Genetics) of expression quantitative trait loci (eQTL) identified *PAM* expression to be associated with rs26232 genotype in multiple tissue types, with the risk C allele associated with lower levels of *PAM* expression. Analysis of the Pathobiology of Early Arthritis database (https://peac.hpc.qmul.ac.uk/) revealed highest levels of *PAM* expression in the fibrous RA synovium compared with lymphoid- or myeloid-rich pathotypes. Single cell RNAseq of RA synovial tissue revealed *PAM* expression to be restricted to RASFs, being greatest in the tissue damaging F1 subtype. siRNA-mediated PAM knockdown resulted in increased RASF proliferation (p = 0.042) and invasion (p = 0.022), and decreased apoptosis (p = 0.01), compared to control siRNA treated RASFs.

Conclusions

This data demonstrates that *PAM* modulates tissue destruction mediated by F1 RASFs. The primary role of PAM is peptide amidation, a post-translation modification that is known to increase the half-life and reactivity of the protein. Additionally, a fragment of the PAM protein enters the nucleus and influences gene expression of a subset of genes by unknown mechanisms. Our ongoing work will concentrate on elucidating the molecular mechanisms by which influences RASF-mediated tissue damage.

rs26232 is Associated with Rheumatoid Arthritis Severity

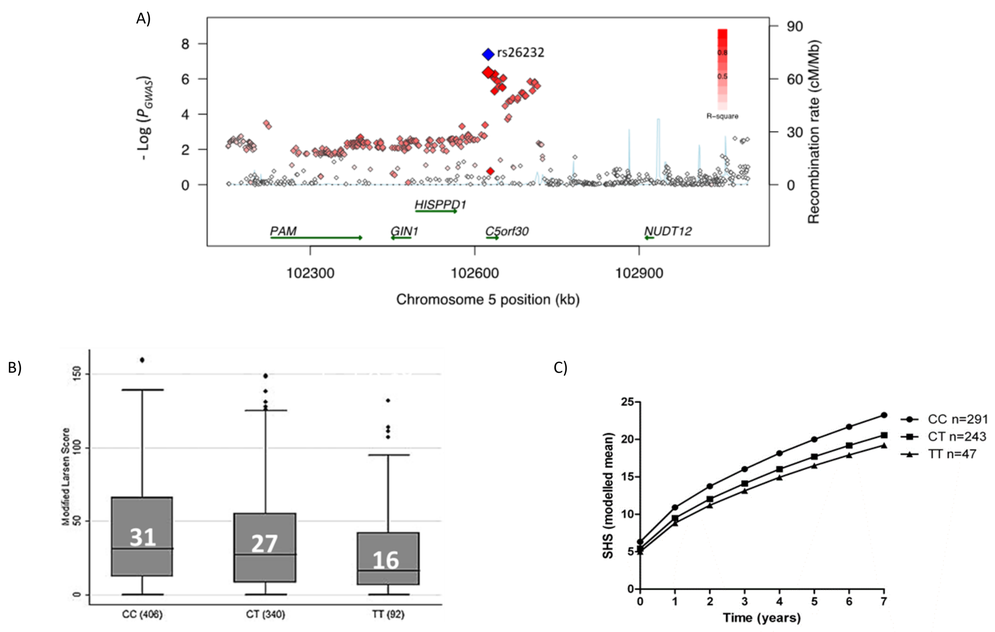

Figure 1: A) Regional association plot showing the strength of association (-Log(P-value)) versus chromosomal position (kilobases, kb) for rs26232[1]. B) Allele-dose association of each genotype of rs26232 with the modified Larsen score of radiologic joint damage in the Genetics of Rheumatoid Arthritis population (Sheffield, UK)[2]. C) Association of each genotype of rs26232 with progression of the modified Sharp/van der Heijde score (SHS) of radiologic joint damage during years 0–7 of follow-up in the Leiden Early Arthritis Clinic cohort[2].

PAM Expression is linked to both rs26232 and RA Severity

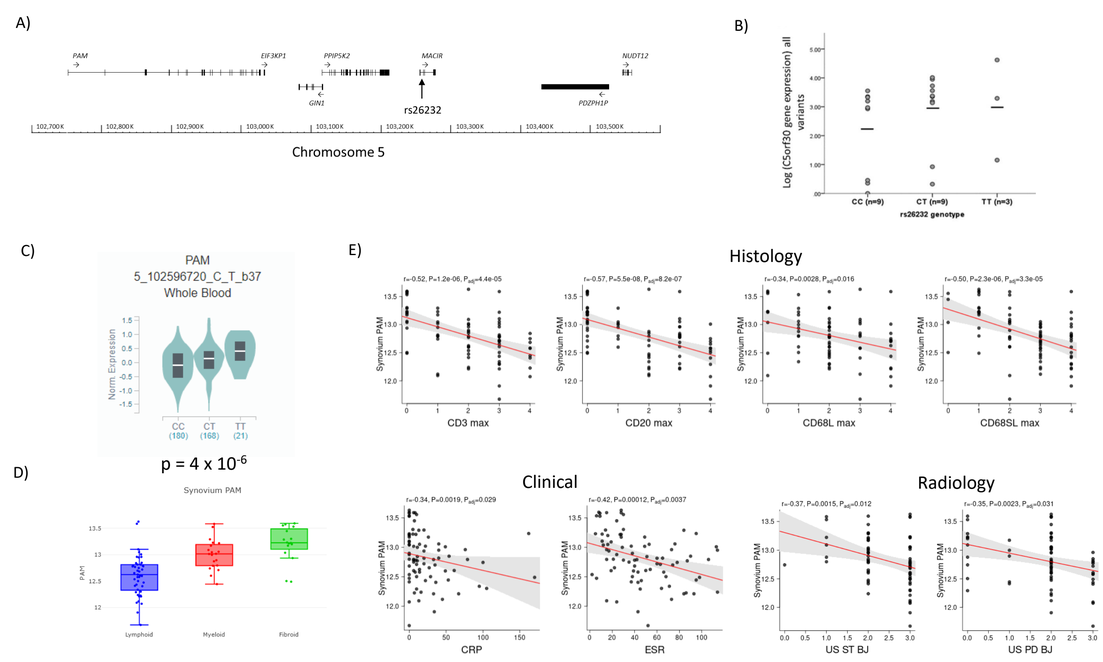

Figure 2: A) Chromosomal map of rs26232 and surrounding genes. rs26232 is located within the first into on *MACIR* B) RT-qPCR data demonstrates that rs26232 genotype is not linked to *MACIR* expression[3] C) Data from GTEx portal demonstrates that rs26232 is an eQTL for *PAM*, with the C allele associated with lower *PAM* expression[4].D) *PAM* expression is highest in fibroid tissue within the RA synovium, compared to myeloid or lymphoid tissue[5]. E)Histological, clinical and radiological markers for RA severity are significantly associated with *PAM* levels, with lower *PAM* expression linked to more severe RA[5].

PAM Expression is Distinctly Expressed in Fibroblasts within the Synovium, Particularly F1-Type

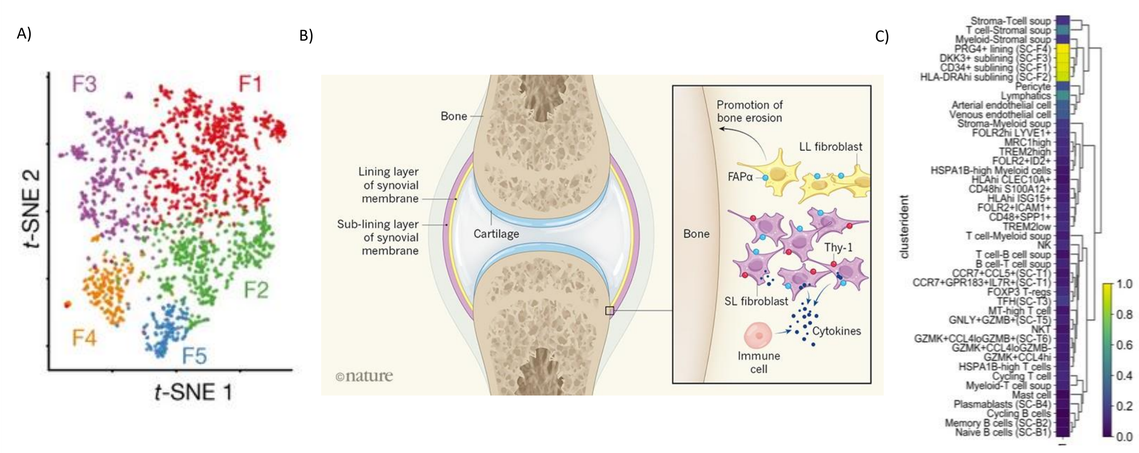

Figure 3: A) Separation of synovial fibroblasts into distinct suptypes by flow cytometry[6]. B) F1 subtype is found in the lining layer and is associated with invasion, bone erosion and joint destruction. Other subtypes contribute to RA pathology through the release of pro-inflammatory cytokines and are found in the sub-lining layer[6]. C) Single cell RNAseq shows *PAM* is distinctly expressed in fibroblasts among the synovial cell population, with highest expression achieved in the F1 subtype.

PAM Knockdown in RASFs Results in a More Aggressive Phenotype

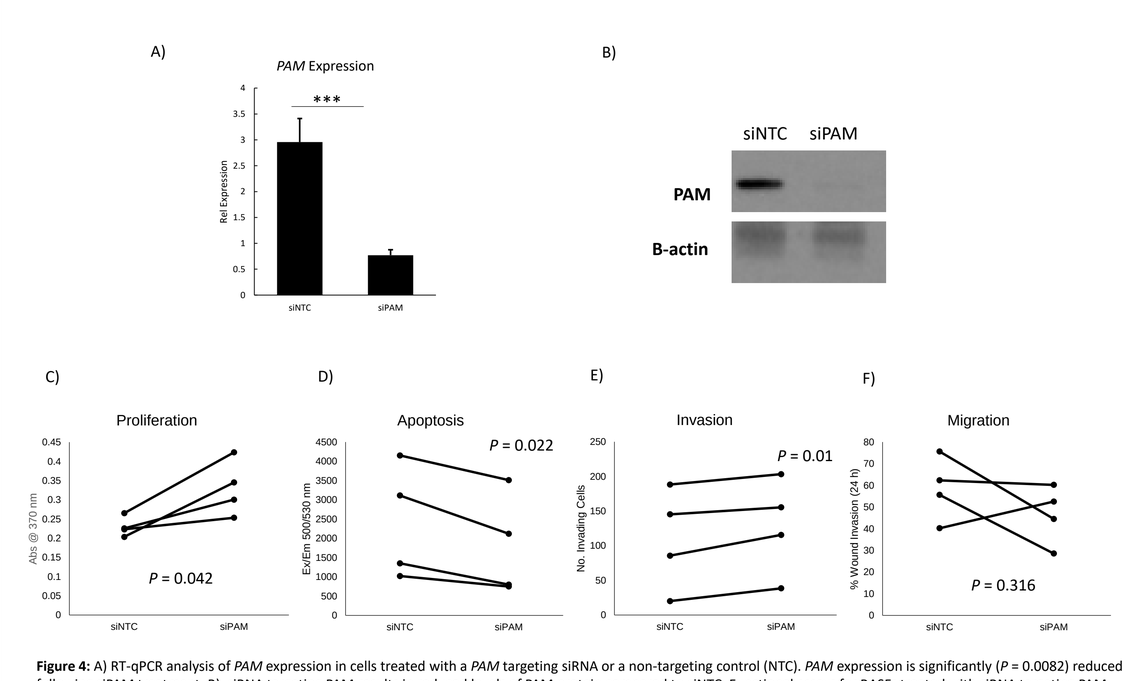

Figure 4: A) RT-qPCR analysis of *PAM* expression in cells treated with a *PAM* targeting siRNA or a non-targeting control (NTC). *PAM* expression is significantly (*P* = 0.0082) reduced following siPAM treatment. B) siRNA targeting PAM results in reduced levels of PAM protein compared to siNTC. Functional assays for RASFs treated with siRNA targeting PAM (siPAM). C) RASFs treated with siPAM show a significantly higher rate of proliferation (*P*=0.042) compared to RASFs treated with non-targeting control siRNA (siNTC). D) RASFs treated with siPAM show a significantly lower rate of apoptosis (*P*=0.022) compared to RASFs treated with siNTC. E) RASFs treated with siPAM show a significantly higher level of invasion (*P*=0.01) compared to RASFs treated with siNTC. F) RASFs treated with siPAM show no significant difference migration levels (*P*=0.316) compared to RASFs treated with siNTC. *P*-values calculated using paired t-test.

Summary

- rs26232 genotype is associated with severity and susceptibility to RA, with the C allele associated with more severe RA.
- rs26232 is located within the first intron of *MACIR*, however *MACIR* gene expression levels are not associated with the rs26232 genotype. This indicates that *MACIR* does not mediate the phenotypes associated with rs26232.
- *PAM* gene expression levels are associated with rs26322 genotype in RASFs, with *PAM* expression highest in TT genotype and lowest in CC. Lower *PAM* expression is linked with an increase in markers for RA. This suggests that the C genotype in rs26232 causes reduced levels of *PAM*, which leads to more severe RA.
- *PAM* is primarily expressed in fibroblasts within the synovial cell population, with highest expression found within the F1 subtype, which causes joint destruction.
- siRNA mediated knockdown of PAM in RASFs results in an increase in proliferation and invasion, while also decreasing apoptosis.
- This data indicates that lower levels of PAM result in a more aggressive phenotype in RASFs and would explain the link between rs26232 genotype and RA.

References

1. Stahl, Eli A., et al. "Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci." Nature genetics 42.6 (2010): 508-514.
2. Teare, M. Dawn, et al. "Allele-dose association of the C5orf30 rs26232 variant with joint damage in rheumatoid arthritis." Arthritis & Rheumatology 65.10 (2013): 2555-2561.
3. Dorris, Emma R., et al. "Association of the Rheumatoid Arthritis Severity Variant rs26232 with the Invasive Activity of Synovial Fibroblasts." Cells 8.10 (2019): 1300.
4. https://gtexportal.org/home/
5. Lewis, Myles J., et al. ""Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes." Cell reports 28.9 (2019): 2455-2470. https://peac.hpc.qmul.ac.uk/
6. Croft, Adam P., et al. "Distinct fibroblast subsets drive inflammation and damage in arthritis." Nature 570.7760 (2019): 246-251.

Acknowledgements

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Contact

Kevin.Sheridan@ucd.ie

rheumatology@ucd.ie

@UCD_CAR

http://www.ucd.ie/car/