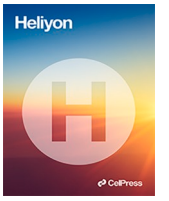
Heliyon 10 (2024) e34937

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

PMEL is a predictive biomarker for mTORC1 inhibitor treatment of renal angiomyolipoma in tuberous sclerosis complex patients

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ARTICLE INFO

Keywords:
PMEL
Tuberous sclerosis complex
Renal angiomyolipoma
Treatment efficacy prediction

ABSTRACT

Background: We aimed to demonstrate the function of premelanosome protein (PMEL) as a biomarker to predict the effectiveness of mammalian target of rapamycin complex 1 (mTORC1) inhibitor treatment in renal angiomyolipomas (RAMLs) in tuberous sclerosis complex (TSC) patients.

Methods: 95 whole blood samples from 49 patients diagnosed with TSC-RAMLs were collected. PMEL, N4BP2, and PCSK1N expression in the plasma samples were tested by quantitative sandwich ELISA. The target tumor volume assessed by maximum cross-sectional area (CSAmax) in CT scans. Correlation analysis was used to determine the relationship between PMEL expression and target tumors, as well as the tumor reduction rate.

Results: The tumor size of TSC-RAMLs positivity correlated with PMEL expression ($r = 0.30$, $p = 0.036$) and PCSK1N expression ($r = 0.23$, $p = 0.027$), but had no significant relationship with N4BP2 ($r = 0.06$, $p = 0.89$). The positive correlation between TSC-RAML tumor volume and PMEL expression still existed in TSC patients before ($r = 0.30$, $p = 0.026$) and after mTORC1 inhibitor treatment ($r = 0.41$, $p = 0.0017$), but the correlation between tumor volume and PCSK1N expression no longer existed. Further analysis found that PMEL expression negatively correlated with the reduction rate of TSC-RAMLs after mTORC1 inhibitor treatment ($r = -0.50$, $p = 0.0022$), both after 3 months ($r = -0.47$, $p = 0.048$) and 6 months of treatment ($r = -0.52$, $p = 0.028$).

Conclusion: PMEL expression positively correlated with the tumor size of TSC-RAMLs, and inversely with the reduction rate of TSC-RAMLs after mTORC1 inhibitor treatment, which may suggest that PMEL may serve as a predictive biomarker for the efficacy of mTORC1 inhibitor treatment.

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https://doi.org/10.1016/j.heliyon.2024.e34937
Received 10 March 2024; Received in revised form 18 July 2024; Accepted 18 July 2024
Available online 25 July 2024

1. Introduction

Tuberous sclerosis complex (TSC) is a rare genetic disorder characterized by mutations in the TSC1 or TSC2 genes, which lead to the upregulation of the mammalian target of rapamycin (mTOR) complex [1]. This disorder is a multiorgan syndrome manifesting with several benign and malignant tumors, with renal lesions present in 80%–85 % of individuals with TSC. Among these, renal angiomyolipomas (RAMLs) are the most prevalent renal abnormalities [2,3]. Moreover, RAMLs are the leading cause of mortality in TSC adult patients due to the risk of life-threatening hemorrhage and are associated with severe chronic kidney disease [4].The updated TSC management recommendations emphasize that mammalian target of rapamycin complex 1 (mTORC1) inhibitors are the primary therapy to preserve renal function and minimize the risk of complications, aligning with the primary therapeutic goal for TSC-related renal angiomyolipomas (TSC-RAMLs) [5]. However, nearly 40 % TSC-RAMLs patients treated with mTORC1 inhibitors fail to reach clinical remission levels (tumor volume reduction of $\geq$50 %) [6,7]. Therefore, we need a simple and effective biomarker to predict the treatment efficacy of mTORC1 inhibitors.

Premelanosome protein (PMEL), also known as gp100 and Pmel17, is predominantly expressed in pigment cells of the skin and eyes. It plays a crucial role in the formation of melanosomal fibrils during the transition from stage I to stage II melanosomes [8]. Recent studies demonstrate that PMEL, as the target of the HMB-45 antibody, has a sensitive and specific expression in angiomyolipomas (AMLs) [9]. Single-cell and single-nuclei RNA sequencing results also shows that PMEL is an expression marker of lymphangioleiomyomatosis (LAM), a metastatic neoplasm associated with TSC gene mutations [10]. Our previous study demonstrated that PMEL has significantly higher expression in TSC-RAMLs and might serve as both prognostic and diagnostic biomarkers [11]. However, whether PMEL can predict the treatment efficacy of mTORC1 inhibitors in TSC-RAMLs requires further clinical validation.

Building upon previous single-cell sequencing and proteomic research, the objective of this study is to demonstrate that whether PMEL could function as a predictive biomarker for monitoring the treatment efficacy of mTORC1 inhibitors.

2. Materials and methods

2.1. Differentially expressed proteins selection in TSC-RAMLs

We reanalyzed our published proteomes data to figure out the upregulated molecules in TSC-RAMLs compared with sporadic angiomyolipoma (S-AML) and renal cysts [11]. The fold change of PMEL, NEDD4 Binding Protein 2 (N4BP2), and Proprotein Convertase Subtilisin/Kexin Type 1 Inhibitor (PCSK1N) protein expression in TSC-RAML relative to S-AML and renal cysts was 29.47 and 4.01, 23.41 and 7.10, 19.64 and 8.97, respectively (all p values < 0.001). Consequently, PMEL, N4BP2, and PCSK1N were identified as prime candidates for further analysis.

2.2. Patients, whole blood samples and clinical data collection

All enrolled patients diagnosed with Tuberous Sclerosis Complex (TSC) were from Peking Union Medical College Hospital between September 2016 and February 2022. Diagnosis was based on the guidelines established by the 2012 International Tuberous Sclerosis Complex Consensus Conference [12]. TSC patients were administered mTORC1 inhibitor medication (either Everolimus at 10 mg/day or Sirolimus at 2 mg/day) orally, with dosage adjustments made according to safety evaluations. Whole blood samples were collected from the included patients before treatment, and at 3 and 6 months of mTORC1 inhibitor treatment. Additionally, abdominal CT scans were performed within one week of each blood sample collection. Whole blood samples were not collected if TSC patients experienced AML bleeding or underwent AML surgical treatments during medication. Plasma from whole blood samples was collected as described in our previous study [11]. The target tumors were assessed using the maximum cross-sectional area (CSAmax) in CT scans (measured in cm^2, calculated by multiplying the maximum diameter of the tumor by the longest diameter perpendicular to it). The size of the target tumor was measured at each follow-up interval. When lesions were present in bilateral kidneys, the CSAmax of TSC-RAMLs was calculated as the average of the maximum lesions on both sides. The efficacy of mTORC1 inhibitors was evaluated based on the reduction rate of CSAmax. All individual participants included in the study provided written informed consent prior to their involvement.

2.3. Quantitation ELISA for protein expression in the plasma samples from TSC-RAMLs patients

For measurement of PMEL, NEDD4 Binding Protein 2 (N4BP2), and Proprotein Convertase Subtilisin/Kexin Type 1 Inhibitor (PCSK1N) expression in the plasma samples from TSC-RAMLs patients, a quantitative sandwich ELISA was developed. PMEL, N4BP2, and PCSK1N expression levels were determined using ELISA kits (NewEast Biotechnology and Cloud-Clone, Wuhan, China) following the manufacturer's protocols. The absorbance of each sample was measured at 450 nm using a microplate reader (BioTek, Winooski, USA), and protein concentrations were calculated based on a standard curve.

2.4. Data analysis

Data analysis was conducted using SPSS 26.0 software (Chicago, IL, USA). The data are presented as mean $\pm$ standard deviation (mean $\pm$ SD) or as n (%) as appropriate. The Shapiro-Wilk test was used to conduct normality tests on the data. If the data followed a normal distribution, we used Pearson correlation analysis for the correlation analysis. Otherwise, we used Spearman correlation

analysis. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Human samples and clinical data

We collected 95 whole blood samples from 49 patients diagnosed with TSC-RAMLs. Among these samples. Of the 95 blood samples, 40 (42.11 %) were collected before mTORC1 inhibitor treatment, and 55 (57.89 %) were collected after mTORC1 inhibitor treatment. Among the 49 included patients, 23 (46.94 %) had baseline data, as well as follow-up data after three or six months of mTORC1 inhibitor treatment. Of the 23 patients, 19 (82.61 %) completed the 3-month treatment follow-up, and 18 (78.26 %) completed the six-month treatment follow-up. Detailed characteristics of the enrolled patients are presented in Table 1.

3.2. PMEL expression positivity correlated with the tumor size of TSC-RAMLs

Subsequent analysis delved into the association between TSC-RAMLs tumor size and the protein expressions of PMEL, N4BP2, and PCSK1N. The findings revealed a significant positive correlation between TSC-RAMLs tumor size and PMEL expression ($r = 0.30$, $p = 0.036$) (Fig. 1A) and PCSK1N expression ($r = 0.23$, $p = 0.027$) (Fig. 1B). Conversely, no significant relationship was observed between N4BP2 ($r = 0.06$, $p = 0.89$) expression and the tumor size of TSC-RAMLs (Fig. 1C). Furthermore, we explored the relationship between TSC-RAMLs tumor size and PMEL expression both before and after mTORC1 inhibitor treatment to eliminate the influence of mTORC1 inhibitors. The results unveiled a positive correlation between PMEL expression and TSC-RAMLs tumor size both before ($r = 0.30$, $p = 0.026$) (Fig. 2A) and after treatment ($r = 0.41$, $p = 0.0017$) (Fig. 2B). However, there was no significant correlation between PCSK1N expression and TSC-RAMLs tumor size both before ($r = 0.10$, $p = 0.24$) and after treatment ($r = 0.55$, $p = 0.97$) (Supplementary Fig. 1).

3.3. PMEL expression negatively correlated with reduction rate of TSC-RAMLs after mTORC1 inhibitor treatment

We then analyzed 23 TSC-RAML patients with comprehensive follow-up data and found that with the extension of drug treatment time, both AML tumor size and PMEL expression decreased (Supplementary Fig. 2). Previous studies proved RAML tumor size as a predictor of mTORC1 inhibitor efficacy in patients with TSC-RAML [13,14]. Therefore, we examined the relationship between the reduction rate of TSC-RAMLs and PMEL expression to determine if PMEL could predict the efficacy of mTORC1 inhibitor treatment. Results showed a negative correlation between PMEL expression and the reduction rate of TSC-RAMLs after treatment, with a correlation coefficient of -0.50 ($p = 0.0022$) (Fig. 3A). Furthermore, we investigated the association between PMEL expression and the reduction rate after 3-month and 6-month treatment. The results indicated PMEL expression exhibited a negative correlation with the reduction rate after 3-month treatment ($r = -0.47$, $p = 0.048$) (Fig. 3B) and 6-month treatment ($r = -0.52$, $p = 0.028$) (Fig. 3C).

4. Discussion

Renal angiomyolipomas (RAMLs) are present in 80 % of patients with Tuberous Sclerosis Complex (TSC), and the rupture and bleeding of renal RAMLs are the leading causes of death among TSC patients [12,15]. Moreover, as RAML increases in size, the risk of rupture and bleeding significantly rises. Therefore, treating RAMLs in TSC patients appears particularly crucial [16]. For patients with TSC-RAMLs, mTORC1 inhibitors could serve as a promising method to manage tumor growth while preserving renal tissue and are recommended as the first-line therapy according to TSC diagnostic criteria and management guideline [16,17]. Moreover, most AMLs

Table 1
Characteristics of enrolled TSC-RAMLs patients.

Characteristics	No. or median (% or range)
TSC-RAML patients (n)	49
Age	30.06 ± 8.47
Sex	
Male	14 (28.57 %)
Female	35 (71.43 %)
RAML lesion location	
Unilateral	22 (44.89 %)
Bilateral	27 (55.51 %)
Patients without follow-up blood samples or CT images (n, %)	26 (53.06 %)
Patients with follow-up blood samples and CT images (n, %)	23 (46.94 %)
after three-month mTORC1 treatment (n)	19 (82.61 %)
after six-month treatment (n)	18 (78.26 %)
Blood samples (n)	95
Before mTORC1treatment (n, %)	40 (42.11 %)
After mTORC1 treatment (n, %)	55 (57.89 %)

RAMLs: renal angiomyolipomas; TSC: tuberous sclerosis complex; mTORC1: mammalian target of rapamycin complex 1 (mTORC1).

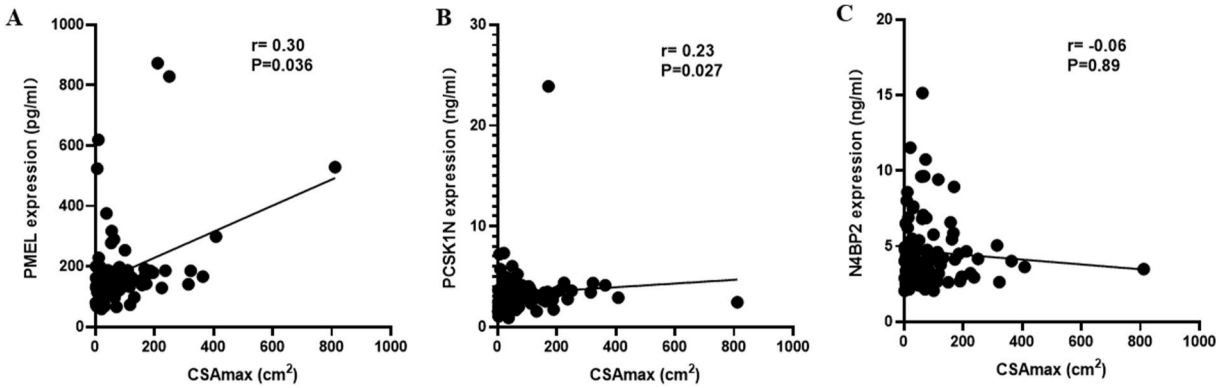

Fig. 1. The relationship between the tumor size of TSC-RAMLs and PMEL (A), PCSK1N (B), and N4BP2 (C) expression. CSAmax: the maximum cross-sectional area of target tumor.

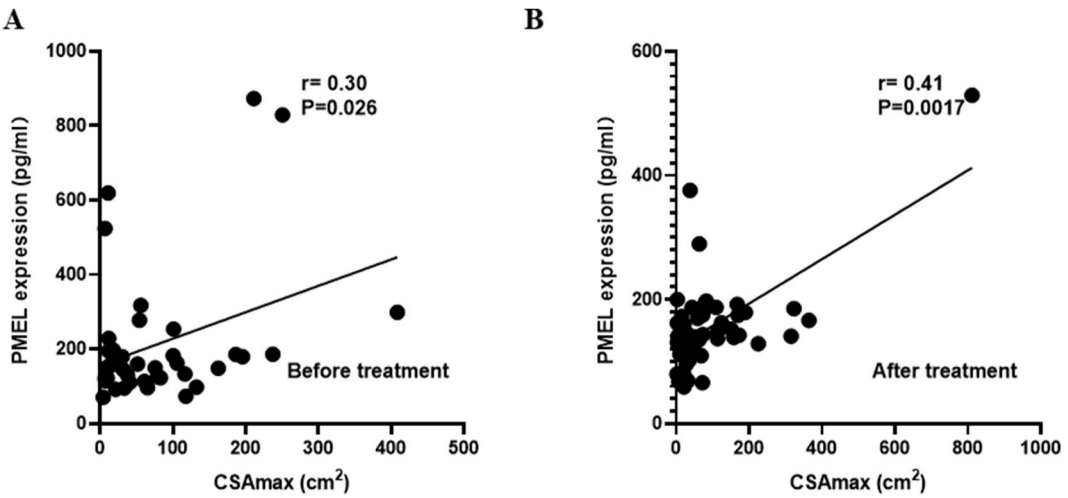

Fig. 2. The relationship between PMEL expression and the tumor size of TSC-RAMLs before (A) and after (B) mTORC1 inhibitor treatment. CSAmax: the maximum cross-sectional area of target tumor.

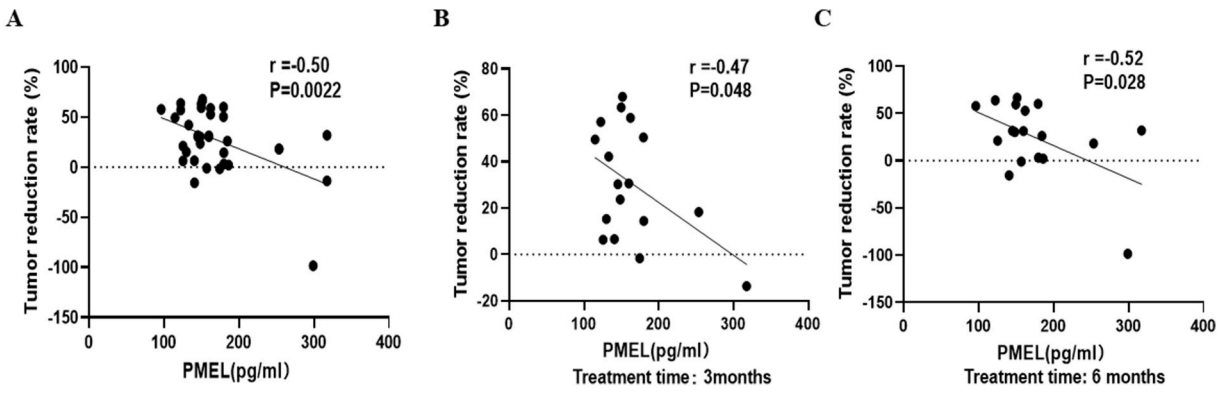

Fig. 3. A: the relationship between PMEL expression and the reduction rate after mTORC1 inhibitor treatment. B: the relationship between PMEL expression and the reduction rate after 3-month mTORC1 inhibitor treatment. C: the relationship between PMEL expression and the reduction rate after 6-month mTORC1 inhibitor treatment.

in TSC patients do not regenerate and rarely bleed during mTORC1 inhibitor treatment, offering long-term clinical benefits [18,19]. Even though mTORC1 inhibitors show promising effectiveness across various clinical trials linked to TSC-RAMLs [18–20], there remains nearly 40–50 % patients still cannot achieve the level of clinical remission (RAML volume reduction rate $\geq$50 %), and approximately 70 % patients suffer from the adverse events of mTORC1 inhibitors [7,21]. Hence, there is a necessity to investigate additional predictive indicators for screening and identifying patients who could potentially benefit from mTORC1 inhibitor therapy.

PMEL, found abundantly in melanosomes, serves as the melanin-producing organelles within melanocytes, playing a crucial role in the structural organization of premelanosomes [22]. Previous studies have found that most cases of pulmonary lymphangioleiomyomatosis (LAM) are caused by the spread of cells from renal AMLs through the bloodstream to the lungs [23,24]. Furthermore, PMEL could be highly expressed in both RAMLs and pulmonary LAM, which were the diagnostic criteria and typical symptoms of TSC patients [10,25]. The main reason might be that both AMLs and pulmonary LAM belong to the family of perivascular epithelioid cell neoplasms (PEComas), originating from perivascular epithelioid cells that express a combination of muscle and melanocytic markers, including HHF-35, PMEL, HMB-50, and others [26,27]. Moreover, immunohistochemistry confirmed the high expression of PMEL in RAMLs, and plasma proteomic and metabolomic profiling using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) also demonstrated the high expression of PMEL in TSC patients [11,26]. Therefore, we aimed to prove that PMEL in plasma can serve as a biological marker for the diagnosis and monitoring of therapeutic progress in TSC-RAML in this study.

Our study firstly proved that PMEL expression positivity correlated with the tumor size of TSC-RAMLs both before ($r = 0.30$, $p = 0.026$) and after mTORC1 inhibitor treatment ($r = 0.41$, $p = 0.0017$). Many studies have proved that TSC patients with larger RAML lesions had poorer response to mTORC1 inhibitor treatment compared to TSC patients with smaller AML volumes, and tumor size was a predictor of mTORC1 inhibitor efficacy in patients with TSC-RAMLs [13,14]. Therefore, we explored the relationship between PMEL expression and the reduction rate of TSC-RAMLs after mTORC1 inhibitor treatment. Results proved that PMEL expression negatively correlated with the reduction rate of TSC-RAMLs after treatment, with a correlation coefficient of -0.50 ($p = 0.0022$), both after 3 months ($r = -0.47$, $p = 0.048$) and 6 months of treatment ($r = -0.52$, $p = 0.028$). The above results demonstrate that PMEL might be a predictive marker for the response to mTORC1 inhibitors in TSC-RAMLs.

However, there are several limitations in our study. Firstly, the number of enrolled patients and blood sample size was relatively small. Secondly, treated patients enrolled in the study received various mTORC1 inhibitors (Sirolimus and Everolimus), potentially introducing bias. However, earlier research has demonstrated the clear efficacy of both Sirolimus and Everolimus in managing TSC-RAMLs, with both being endorsed as primary therapies [21,28,29]. The predictive function of PMEL as proved by our study might extend to a broader population of TSC patients undergoing treatment with Sirolimus or Everolimus. Thirdly, we designated the largest TSC-RAML in kidneys as the target tumor for analysis, potentially introducing specific biases when extrapolating findings to evaluate all tumors. The specific mechanism by which PMEL predicts the shrinkage of tumors induced by mTORC1 inhibitors also requires further investigation.

5. Conclusion

The positivity of PMEL expression correlated with the tumor size of TSC-RAMLs both before and after treatment with mTORC1 inhibitor, and inversely correlated with the reduction rate of TSC-RAMLs after mTORC1 inhibitor treatment. PMEL could serve as a predictive marker for the response to mTORC1 inhibitors in TSC-RAMLs.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Peking Union Medical College Hospital (I-23PJ093). Written informed consent was obtained from all individual participants included in the study.

Funding

This study was funded by the grant from National High Level Hospital Clinical Research Funding (2022-PUMCH-A-151).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

All authors approved the manuscript.

CRediT authorship contribution statement

Dongxu Qiu: Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Zhan Wang:** Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Xu Wang:** Visualization, Validation, Software, Resources, Data curation. **Yutao Wang:** Writing – review & editing, Validation, Methodology, Investigation. **Wenda Wang:** Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Yushi Zhang:** Project administration,

Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thank China Scholarship Council (CSC) for funding Dongxu Qiu's studies in University of British Columbia, Canada.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34937.

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