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Original Article

Expression of peroxisome proliferator-activated receptor (PPAR) family and survival analysis in patients with multiple myeloma using microarray gene expression profiles

Aikebaier Maimaiti[1], Yong-Qin Zhao[1], Zhang Ting[1], Wei Huang[2], Linsen Shi[1]

[1]Zhejiang Chinese Medical University, Hangzhou 311402, Zhejiang, China; [2]Division of Hematologic Malignancies and Cellular Therapy, Duke University, Durham, USA

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Abstract: Peroxisome proliferator-activated receptor (PPAR) family consists of 3 members: alpha, gamma, and delta (beta). PPARs play essential roles in cellular differentiation, development, and metabolism. However, little is known about the impact of PPARs on myeloma progression. The aim of this study is to systematically investigate the expression and survival rate of PPARs in myeloma. Using the public available datasets downloaded from Gene Expression Omnibus (GEO), gene expression of PPARs in clinical samples from human bone marrow and myeloma was obtained. Meanwhile, the prognostic value of PPARs in the primary myeloma was assessed using the Arkansas and Mulligan myeloma microarray datasets. mRNA levels of PPARs have a relatively lower expression in myeloma compared to bone marrow cells. High expression of PPARs predicts a good prognostic value for the overall survival rate. In conclusion, in this study, we elaborate the role of PPARs in myeloma progression, which may provide useful clinical significance for target/drug development for myeloma therapeutics.

Keywords: PPARs, myeloma, expression analysis, prognostic value, microarray expression analysis

Introduction

Multiple myeloma (MM), also known as plasma cell myeloma, is a cancer of plasma cells originally formed by malignant plasma cells [1]. From 2011 to 2013, there were an estimated 11,200 new MM cases per year in men and 8,500 new cases per year in women. By the end of 2016, it is estimated that 12,590 deaths (6,660 men and 5,930 women) occurred from this disease. The 5-year survival rate for people with multiple myeloma is about 49% [2]. Myeloma arises from an asymptomatic premalignant proliferation of monoclonal plasma cells that are derived from post-germinal-center B cells [1]. Several genetic abnormalities that occur in tumor plasma cells play major roles in the pathogenesis of myeloma, such as *MMSET, FGFR3* [3] *and MYC* [4] *etc*. Besides, alteration in microRNAs expression and gene methylation modifications also contributed to myeloma progression [5].

Gene expression microarrays provide a snapshot of all the transcriptional activity in a biological sample. Unlike most traditional molecular biology tools, which generally allow the study of a single gene or a small set of genes, microarrays facilitate the discovery of totally novel and unexpected functional roles of genes. The power of these tools has been applied to a range of applications, including discovering novel disease subtypes, developing new diagnostic tools, and identifying underlying mechanisms of disease or even drug response [6, 7].

The peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes [8]. PPARs play essential roles in cell differentiation, development and metabolism [9], and have been shown to participate in tumorigenesis recently [10]. However, little is known about their impacts on myeloma. To systematically under-

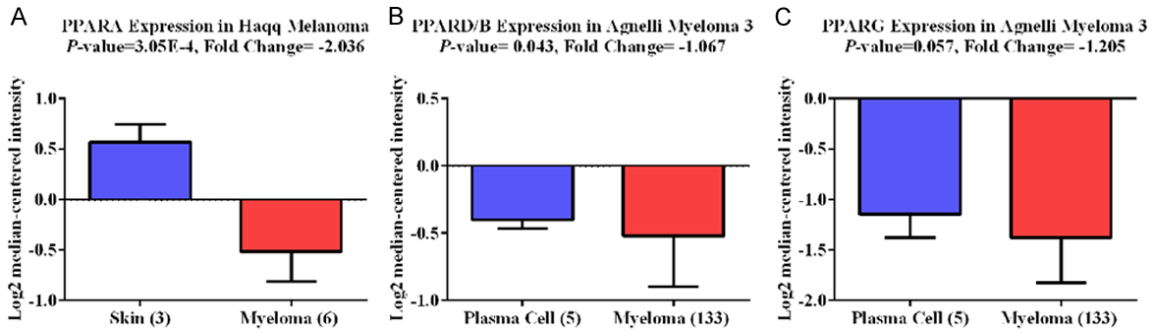

Figure 1. mRNA levels of PPAR gene family in patients with myeloma. A. mRNA level of *PPARA* in myeloma dataset obtained from Haqq myeloma. B. mRNA level of *PPARD* in myeloma dataset obtained from Agnelli myeloma. C. mRNA level of *PPARG* in myeloma dataset obtained from Agnelli myeloma. Expression levels are presented as boxplots and were compared using an unpaired Student's *t* test.

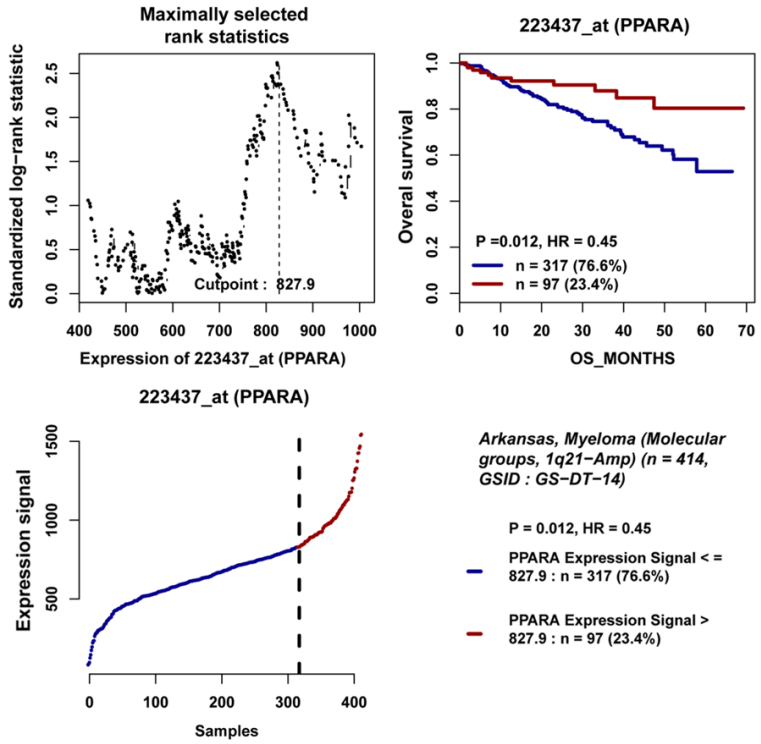

Figure 2. Overall survival analysis of *PPARA* in the Arkansas dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

stand the role of PPARs, in this study, we investigate the expression level of PPARs in MM using the publicly available datasets downloaded from Gene Expression Omnibus (GEO) database. Besides, survival analysis in terms of PPAR family members also validated using Arkansas and Mulligan myeloma microarray datasets. According to our expression analysis, mRNA level of PPARs was decreased in MM patients compared to the vehicle control bone marrow cells, illustrating their potential role of tumor suppressor in MM progression. In addition, high levels of PPARs in MM patients predict a good survival rate. More detailed information about our experimental setup is given below.

Material and methods

Gene expression dataset

All the microarray datasets utilized for PPARs gene expression were downloaded from Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/) with a series matrix File (.txt). Datasets were processed basing on log2 transformation before conducting the expression analysis.

Clinical value of PPARs in myeloma patients

Overall survival (OS) of PPARs in myeloma patients was accessed using a data set of patients originally derived from Arkansas [11] and Mulligan myeloma [12] microarray as previous described [7].

Statistical analysis

For expression analysis, statistical analysis was performed using two class paired stu-

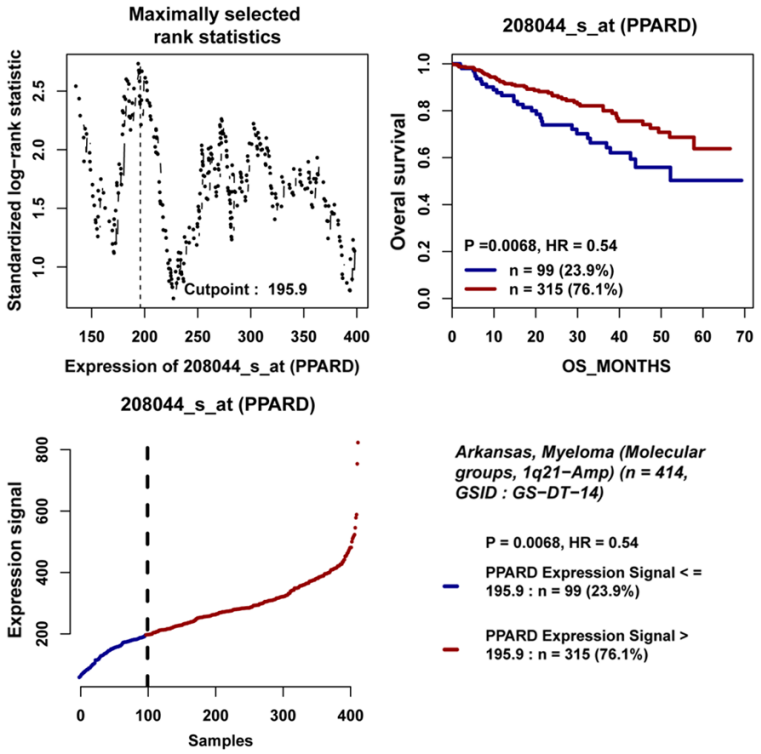

Figure 3. Overall survival analysis of *PPARD* in the Arkansas dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

dent's t test. Gene expression was considered to be significant if the threshold of *p* value less than 5%. For survival analysis, Kaplan-Meyer analysis of the over-all survival was performed in the Arkansas and Mulligan myeloma microarray datasets based on *the PPARs gene expression. Survival analysis was performed using a log-rank test. *p < 0.05, **p < 0.01 and ***p < 0.001 (one-way ANOVA with Tukey's post-test).*

Results

PPARs gene family functioned as tumor suppressors in myeloma progression

To better understand the role of PPARs in myeloma progression, firstly, Haqq myeloma microarray dataset was used for *PPARA* expression analysis [13]. As shown in **Figure 1A**, mRNA level of *PPARA* decreased from 0.567 to -0.517 (median), suggesting it functioned as a tumor suppressor in myeloma progression. Besides, we also analyzed mRNA level of *PPARD* (also called *PPARB*) in Agnelli myeloma, which derived from purified plasma cells obtained from 5 normal donors and 133 multiple myeloma [14]. **Figure 1B** indicated that *PPARD* had a relatively lower expression in myeloma cells compared to the vehicle control. In addition, expression level of *PPARG* was also verified using Agnelli myeloma and it was found to be decreased dramatically as shown in **Figure 1C**. In conclusion, our expression analysis of PPARs gene family (*PPARA*, *PPARD* and *PPARG*) seemed to support the beneficial role of PPARs in myeloma initiation and progression.

High expression of PPARs gene family predicts a good prognostic value in myeloma

To further determine the prognostic value of PPARs expression in primary myeloma, Arkansas and Mulligan myeloma microarray datasets were used for survival analysis. As shown in **Figures 2**-**4**, the Arkansas dataset suggests that the overall survival rate is higher in patients with high PPARs expression, compared to those patients with low *PPARA* (**Figure 2**), *PPARD* (**Figure 3**) and *PPARG* expression (**Figure 4**). Despite the separation or the difference between the two populations in terms of *PPARG* was not significant, we can still see the similar trends as shown in *PPARA* and *PPARD*. In addition, the Mulligan dataset revealed that myeloma patients with lower PPARs mRNA were significantly associated with decreased survival time (**Figures 5**-**7**). These findings showed the clinical significance for PPAR gene family in myeloma and validated the need to further understand the regulation of PPARs expression and function.

Discussion

With the help of high-through Screening (HTS) analysis, determination of gene expression for cells and tissue has become a major tool for scientific research in medicine [15-17]. Microarray experiments allow description of genome-wide expression changes in health and disease. The results of such experiments

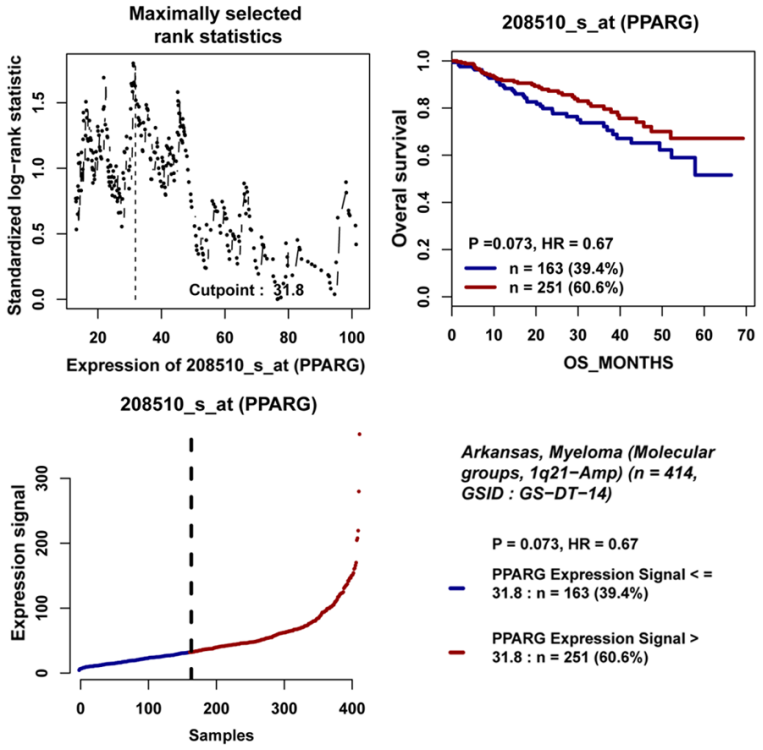

Figure 4. Overall survival analysis of *PPARG* in the Arkansas dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

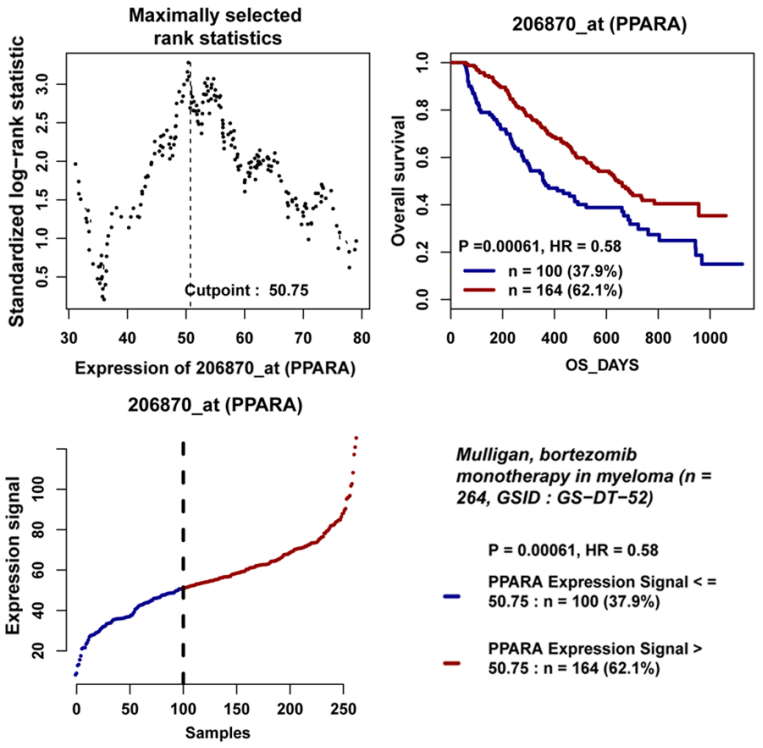

Figure 5. Overall survival analysis of *PPARA* in the Mulligan dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

are expected to change the methods employed in the diagnosis and prognosis of disease, especially for cancers [18]. In the study, the microarray analysis confirmed that PPARs were decreased in myeloma. As ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, three subtypes of PPARs have been identified to be highly associated with cancer progression [19]. In some tissues, the expression level of PPARs and/or their activation correlates with a positive outcome against cancer, while, in other tissue types, their expression and activation have the opposite effect [20]. To determine if PPARs expression is of clinical relevance, we interrogated databases of gene expression in patient samples. Multiple independent datasets of patient samples indicated that PPARs expression was decreased in the primary tumors of patients with lung, liver and brain cancers. This is consistent with previous publications. In brain tumor, some studies have shown that PPARγ agonists interfere with glioblastoma growth and malignancy, as well as inhibit growth and expansion of brain tumor stem cells [21, 22]. For lung cancer, PPARα activation generally inhibits tumorigenesis through its antiangiogenic and anti-inflammatory effects [23]. Pharmacological activation of PPARs could attenuate lung cancer [24, 25]. Other anti-cancer effects of PPARs ligands have been reported in several gastric cancer [26], liver cancer [27], pancreatic cancer [28] *etc*. As an anti-inflammatory nuclear receptor, Otsuyama and colleagues explored the expression level

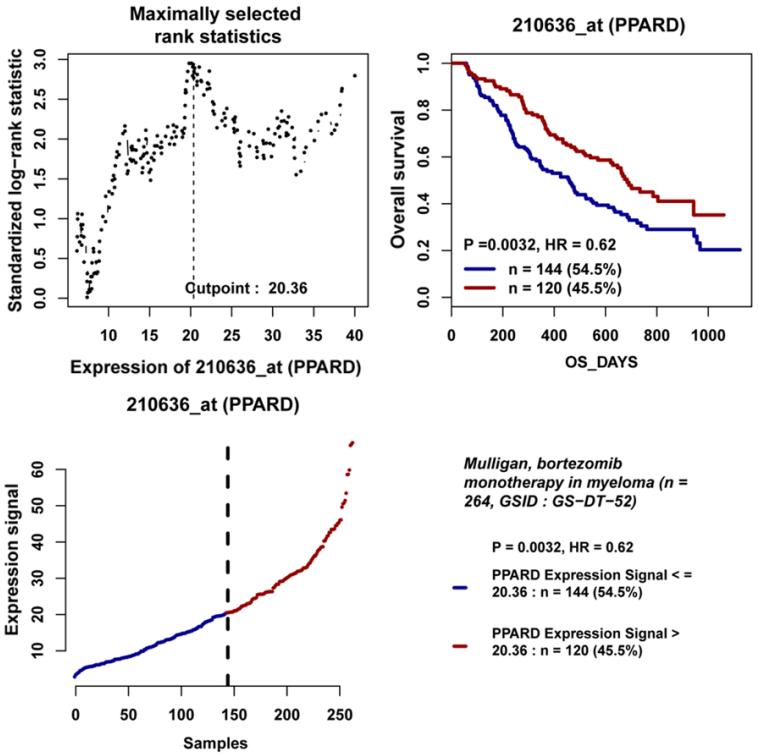

Figure 6. Overall survival analysis of *PPARD* in the Mulligan dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

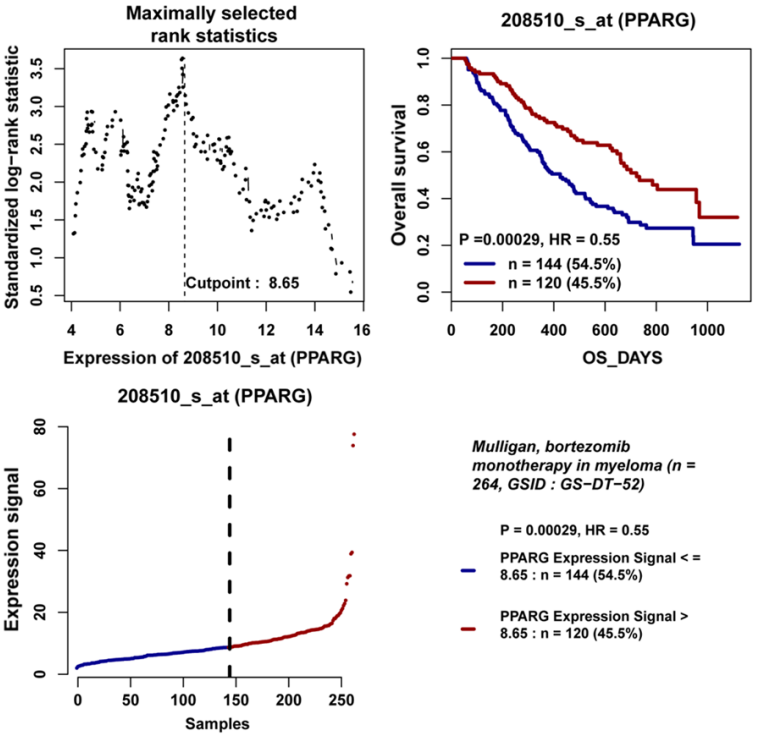

Figure 7. Overall survival analysis of *PPARG* in the Mulligan dataset. Survival analysis was performed using a log-rank test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

of PPARs in myeloma and whether PPAR activation could inhibit the proliferation of myeloma cells *in vitro* [29]. Finally, authors concluded that primary myeloma cells from newly diagnosed MM patients as well as myeloma cell lines predominantly expressed the PPARβ gene and also showed the weak expression of PPARγ. And activation of PPARβ and PPARγ using carbacyclin and troglitazone would result in the suppressive effect on primary myeloma cells as well as myeloma cell lines. Thus, these studies confirmed the tumor suppressor role of PPARs in myeloma, which is consistent with our expression analysis.

To test the prognostic value of PPARs expression in Myeloma, we used two microarray datasets from Arkansas and Mulligan. The Arkansas dataset available indicated that high PPARs mRNA in the tumors of myeloma was associated with longer time to relapse. Despite the separation or the difference between the two populations in terms of *PPARG* was not significant, we can still see the similar trends as shown in *PPARA* and *PPARD*. Besides, the Mulligan dataset also confirmed that low expression of PPARs was significantly associated with decreased survival and shorter time to relapse, which was validated by previous groups [29].

In conclusion, in this study, we explored the expression of PPARs (α, β and γ), and test the prognostic value of PPARs in patients with myeloma. Importantly, our data revealed that PPAR gene family served as tumor suppressors in myeloma initiation and pro-

gression and could be used as a predictor of cancer progression in patients suffering from myeloma.

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Disclosure of conflict of interest

None.

Address correspondence to: Linsen Shi, Collge of Pharmaceutical Science, Zhejiang Chinese Medical University, Gaoke Road, Fuyang District, Hangzhou 311402, Zhejiang, China. Tel: +86-0571-61768135; Fax: +86-0571-61768135; E-mail: pjstone@163.com

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