



RESEARCH ARTICLE

REVISED The association between FOXO3a rs4946936 gene polymorphism and the levels of FOXO3a among chronic granulocytic leukemia patients treated with imatinib mesylate [version 3; peer review: 2 approved]

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Abstract

Background: The gene *FOXO3a* has been elucidated to govern the development of chronic granulocytic leukemia (CGL). Moreover, it has been suggested that the levels of *FOXO3a* in circulation are affected by the *FOXO3a* rs4946936 gene polymorphism. However, no study has assessed the correlation between the *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a*. The objective of this study was to assess the association between the *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* in CGL patients treated with imatinib mesylate.

Methods: A cross-sectional study was conducted from February 2019 to February 2020. The genotyping of *FOXO3a* rs4946936 gene polymorphism was conducted using PCR-RFLP, and the levels of *FOXO3a* were assessed using ELISA. The association between the *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* were assessed using multiple logistic regression.

Results: A total of 60 CGL patients were assessed in our study. Among them, the CC, CT, and TT genotypes of the *FOXO3a* rs4946936 gene

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1. **Kazuhiro Naka** , Hiroshima University, Hiroshima, Japan

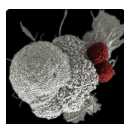
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polymorphism were 35.0%, 48.3%, and 16.7% respectively. Our calculation revealed that elevated levels of *FOXO3a* were found in CGL patients with the CC genotype of the *FOXO3a* rs4946936 gene polymorphism. While we failed to clarify the association between either the CT or the TT genotype of *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a*.

Conclusion: Our study identifies that the CC genotype of the *FOXO3a* rs4946936 gene polymorphism affects the elevated levels of *FOXO3a* in CGL patients treated with imatinib mesylate.

Keywords

chronic granulocytic leukemia, FOXO3a, FOXO3a rs4946936 gene polymorphism



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REVISED Amendments from Version 2

In the version 3, we provide the revision of Introduction. The revision does not change the main findings of our study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Chronic granulocytic leukemia (CGL), first recognized in 1845, is a myeloproliferative disease caused by a condition in which a single pluripotential haemopoetic stem cell acquires the Philadelphia chromosome.¹ A global report in 2015 revealed that the incidence of this disease was estimated between 0.7 and 1.0 per 100,000 inhabitants,² while mortality was reported at less than 15%.³ The pathogenesis of CGL is a comprehensive process, and may involve a wide variety of biomarkers including cyclooxygenase 1 (*COX1*), patched homolog 1 (*PTCH1*), Forkhead Transcription Factor 3a (*FOXO3a*), prostaglandin-endoperoxide synthase 1 (*PTGSI*), and human organic cation transporter 1 (*hOCT1*).^{4–6} Of them, *FOXO3a* is proposed as one biomarker having a pertinent role in the pathogenesis of CGL, although evidence is limited.⁶

FOXO3a belongs to the FOXO Forkhead transcription factors subfamily. The FOXO transcription factors have a various function in the cell stability, including the activation of target – gene expression and the inhibition of target – gene expression, depending on the type of their subfamily and their interaction to the specific protein.⁷ The subfamily of FOXO includes *FOXO1*, *FOXO3a*, *FOXO3b*, *FOXO4* and *FOXO6*.⁸ Of them, *FOXO3a* plays a dominant role to activate the expression of target gene in the pathogenesis of CGL, and may contribute to the progression of CGL.^{6,7,9} Moreover, the existence of *FOXO3a* in the circulation is governed by the *FOXO3a* gene, which has several single nucleotide polymorphisms (SNPs) such as rs2802292, rs2764264, rs4945816, rs9400239, rs4946936, and rs13217795.¹⁰ Of them, the *FOXO3a* rs4946936 gene variant is suggested to have a crucial role in affecting the levels of *FOXO3a* in circulation, and therefore may have a potential role in the pathogenesis of CGL. While the gene polymorphism of *FOXO3a* rs4946936 has been investigated in the case of vitiligo,¹¹ chronic obstructive pulmonary disease (COPD),¹² acute lymphoblastic leukemia,¹³ thyroid cancer,¹⁴ and hepatocellular carcinoma,¹⁵ the role of *FOXO3a* rs4946936 gene polymorphisms in the development of CGL has never been examined. Therefore, we aimed to assess the correlation between *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* in patients with CGL treated with imatinib mesylate. Our report may serve as an initial insight into the role of the *FOXO3a* rs4946936 gene polymorphism in the pathogenesis of CGL.

Methods**Study design and patients**

During the period from February 2019 to February 2020, we conducted a cross-sectional study in Saiful Anwar General Hospital, Malang, Indonesia. A total sampling method was applied to recruit the study participants (a total of 26 participants was needed as the minimum sample size according to the estimation that the prevalence of CGL was 10–12 per 100,000 inhabitants with a 5% margin of error and 95% confidence level). The patients were included in our study if they met the following inclusion criteria: (1) all CGL patients (confirmed by a positive Bcr-ABL test) treated with imatinib mesylate for at least six months in our hospital during the study period, (2) aged more than 18 years old, and (3) willing to participate in the study, proven by giving written informed consent. Patients were excluded from our study if they had a history of the following conditions: prostate cancer, gynecological cancer, COPD, breast cancer, vitiligo, and thyroid cancer.¹⁶ Our present study was conducted following the ethics code of the Helsinki Declaration and was registered and approved by the Ethical Committee of Universitas Brawijaya (No. 400/098/k.3/302/2019).

Genotyping of *FOXO3a* rs4946936 gene polymorphism

We collected venous blood in plastic vacutainer tubes containing EDTA. We separated the blood samples, and they were stored at -20°C . We centrifuged the blood samples in EDTA at $1000 \times g$ for 10 min, and we separated the plasma and stored it at -20°C until it was used to measure *FOXO3a* levels. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to genotype the *FOXO3a* rs4946936 gene polymorphism. The procedures in *FOXO3a* genotyping including the primer used, amplification, and PCR cycles (initial denaturation, denaturation, annealing, and extension) were adapted from previous studies,^{11,12} using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, California, USA). The *FOXO3a* primer (*FOXO3a* F: 5'-GGGTCCTGAGAACTTCTGAGT-3'; *FOXO3a* R: 5'-GACATTCTGTAAGACATTCTGCCT-3') used in our present study was MyTaq HS Mix, 2X (Bioline, Meridian Bioscience™, OH, USA). We used SfcI (New England Biolabs, Massachusetts, USA) as the restriction enzyme. PCR was conducted in a 50 μl volume with 20–100 ng DNA, 20 pmol of each primer, 50 μM KCl, 100 μM dNTPs, 20 μM Tris-HCl pH 8.6.

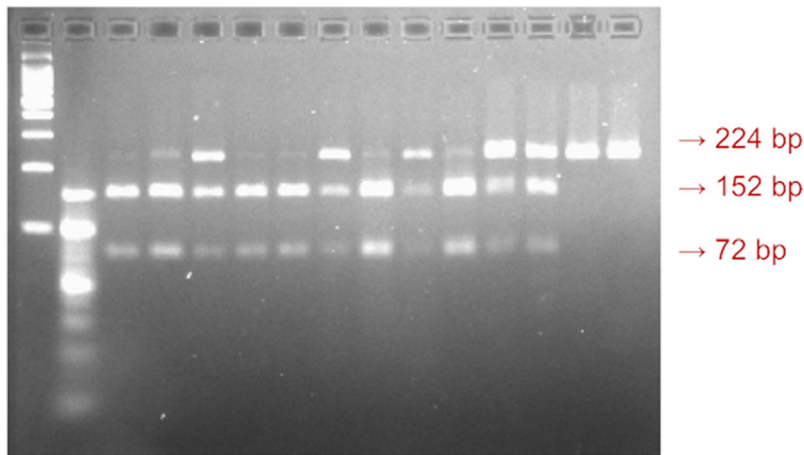


Figure 1. A visualization of the *FOXO3a* rs4946936 gene polymorphism. The 224bp sized band indicated TT genotype, the 152bp size band indicated CT genotype, and the 72bp sized band indicated CC genotype.

1 mM MgCl₂, and 1 U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). Amplification was carried out using an automated thermal cycler (Techne-Genius, Princeton, NJ). The PCR cycles consisted of initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 7 min. The amplified products were digested with one unit of SfiI (rs4946936; C/T). The digested fragments were separated on 2% agarose gel by electrophoresis. The genotypes were identified according to their fragment sizes. The TT genotype was characterized by 224 bp sized bands, the TC genotype was characterized by 224, 152, and 72 bp sized bands, and the size bands of 152 and 72 indicated the CC genotype (Figure 1).

The measurement of *FOXO3a* levels

The measurement of serum *FOXO3a* levels was conducted by the enzyme-linked immunosorbent assay (ELISA) method, using the Cusabio kit (Cusabio Biotech Co., New York, USA), with the procedures conforming to the manual instructions from the company. The 100 µl standard liquid and blood sample were added to the tubes, and incubated for 90 min. The liquid from each tube was then shaken. The 100 µl biotinylated detection antibody was added to each tube and incubated at 37 °C for 1h. The liquid of each tube was shaken and washed three times. The 100 µl 1x HRP conjugate was then added to each tube and incubated at 37 °C for 30 min. The liquid was shaken and washed five times. The 90 µl TMB substrate was added to each tube and incubated at 37 °C for 15 min. The 50 µl stopper liquid was added to each tube. At this step, a change from blue to yellow might occur. Measurement of optic density at 450 nm was conducted. The results were then interpreted to the standard curve to determine the levels of *FOXO3a* and expressed in units of pg/mL.

Statistical analysis

The correlation between the *FOXO3a* rs4946936 gene polymorphism and *FOXO3a* levels was assessed using multiple logistic regression. A p-value of less than 0.05 indicated a significant association. The effect estimates between groups were presented by calculation of the mean difference. We used the R stats package statistical software to analyze the data in our study (R Project for Statistical Computing, RRID:SCR_001905).

Results

Baseline characteristics

A total of 60 CGL patients were analyzed. Of them, 21 (35.0%), 29 (48.3%), and 10 (16.7%) patients had a CC, CT, and TT genotype, respectively. Initially, we employed a total of 68 CGL patients, however, eight patients were excluded due to having a history of gynecological cancer and COPD. Table 1 describes the baseline characteristics of patients included in our study. The detailed data are presented as underlying data.¹⁷

Main findings

Our findings identified that elevated levels of *FOXO3a* were observed among CGL patients with CC (CC vs. CT + TT) genotype (MD: 26.56; 95% CI: 0.48, 52.64). While the association between the CT (CT vs. CC + TT) and TT (TT vs. CC + CT) genotypes and the levels of *FOXO3a* were unverified (Figure 2).

Table 1. Baseline characteristics of chronic granulocytic leukemia patients treated with imatinib mesylate included in the study examining the association between FOXO3a rs4946936 gene polymorphism and the levels of FOXO3a.

Characteristics	FOXO3a gene polymorphism			p
	CC	CT	TT	
Gender				
Male	11 (18.30)	15 (25.00)	5 (8.30)	31 (51.70)
Female	10 (16.70)	14 (23.30)	5 (8.30)	29 (48.30)
Age	43.62 ± 11.75	41.9 ± 12.53	43.4 ± 14.18	10.12 ± 4.04
Spleen				
S3	4 (6.70)	4 (6.70)	0 (0.00)	8 (13.30)
S4	6 (10.00)	7 (11.70)	4 (6.70)	17 (28.30)
S5	5 (8.30)	13 (21.70)	5 (8.30)	23 (38.30)
S6	6 (10.00)	5 (8.30)	1 (1.70)	12 (20.00)
Hemoglobin	11.59 ± 4.18	10.9 ± 3	10.12 ± 4.04	11.01 ± 3.6
Leucocyte	25698.1 ± 56533.51	42165.55 ± 94168.6	138092 ± 162617.05	52389.68 ± 104285.9
Thrombocyte	218047.62 ± 102640.38	423241.38 ± 963616.32	306900 ± 183752.52	332033.33 ± 676914.46

Note: Total no. of patients = 60. Data were presented as mean ± SD or no. (%).

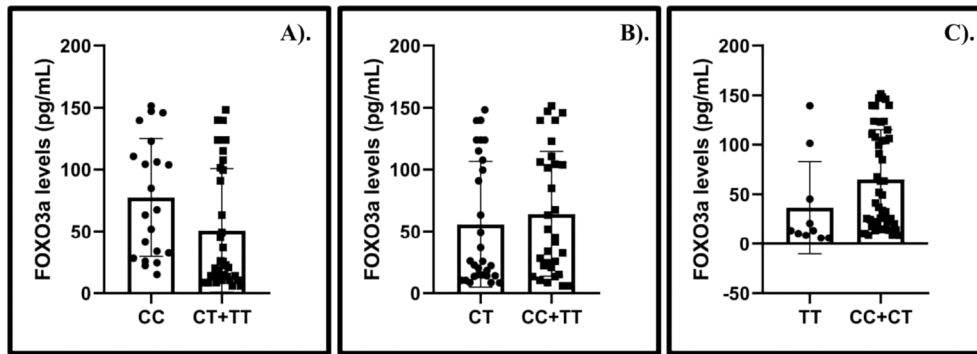


Figure 2. The summary of *FOXO3a* levels and the gene polymorphism of *FOXO3a* in chronic granulocytic leukemia patients treated with imatinib mesylate. A). CC vs. CT+TT, MD: 26.56, 95%CI: 0.48, 52.64, p: 0.0460; B). CT vs. CC+TT, MD: -8.45, 95%CI: -34.09, 17.19, p: 0.5180; C). TT vs. CC+CT, MD: -28.33, 95%CI: -62.06, 5.39, p: 0.1000.

Discussion

Our study identified that the CC genotype of the *FOXO3a* rs4946936 gene polymorphism was associated with increased levels of *FOXO3a* among patients with CGL treated with imatinib mesylate. The study in the context of the association between the *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* in patients with CGL treated with imatinib mesylate had, to our knowledge, never been performed previously, therefore, an holistic comparison including the gene-ethnicity or gene-environment interaction could not be discussed. Previous studies in this context had been performed in the case of COPD¹² and vitiligo.¹¹ In the case of vitiligo, a study failed to clarify the correlation between the levels of *FOXO3a* and the gene polymorphism of *FOXO3a* rs4946936.¹¹ Moreover, in the case of COPD, no association was observed between the levels of *FOXO3a* and the gene polymorphism of *FOXO3a* rs4946936.¹² The different findings between our study in the case of CGL and previous studies in the case of vitiligo and COPD are contradictory and debatable, and a possible reason might be proposed. Briefly, vitiligo and COPD are known as conditions that affect the levels of *FOXO3a*, and the mechanism of *FOXO3a* production in the case of CGL might differ to the case of vitiligo and COPD. In the case of vitiligo, *FOXO3a* may interrupt the oxidative pathway by controlling the target gene expression.¹¹ In the case of COPD, *FOXO3a* may stimulate the expression of Atrogin-1/MAFbx, a biological marker having a pivotal role in the development of COPD.¹² On the other hand, the role of *FOXO3a* in the case of CGL occurs through establishing the phosphorylation by p210BCR-ABL tyrosine kinase, and may contribute to the proliferation of leukemic progenitors.^{18,19}

Theoretically, the association between the levels of *FOXO3a* and the gene polymorphism of *FOXO3a* rs4946936 remain conflicting. However, several mechanisms might be proposed. First, the gene-gene interaction or gene-protein interaction might underly our findings. This possible mechanism was supported by a previous study.²⁰ The authors revealed that a dominant homozygote genotype of the *FOXO3a* rs4946936 gene variant was reported to associate with elevated levels of immunoglobulin (Ig) E in the case of asthma. They proposed that *FOXO3a* might regulate the production of proinflammatory cytokine, and cause elevated levels of IgE.²⁰ Second, free energy levels between genotypes of the *FOXO3a* rs4946936 gene polymorphism might affect the levels of *FOXO3a* in the circulation. In an *in silico* study, the free energy levels of the CC and TT genotype of the *FOXO3a* rs4946936 gene variant were -320.80 Kcal/mol and -301.10 Kcal/mol, respectively, indicating that the CC genotype had lower free energy levels.²¹ Low free energy levels were associated with the mRNA structure and translation, and the changes of mRNA translation at the 5' and 3' UTR locations were correlated to affect protein synthesis.²¹⁻²³ This explanation might describe the possible underlying mechanism that the CC genotype of the *FOXO3a* rs4946936 gene variant had an association with increased levels of *FOXO3a* in the case of CGL compared to the CT and TT genotype. Moreover, previous study also supported our findings. They found that elevated levels of *FOXO3a* was associated with treatment failure of CGL patient treated with imatinib mesylate, suggesting that the levels of *FOXO3a* might play an important role to contribute the progression of CGL.¹⁶ Furthermore, studies also found that elevated levels of *FOXO3a* was associated with adverse prognosis of leukemia.^{24,25} However, gene - environment interaction should also be considered to affect the disease progression since it was proven that the FOXO transcription factors had various impact in different population, for example: the role of FOXO transcription factors were found inconclusive to affect the human longevity in the population of Japan, Germany, Italia, and Indonesia.^{26,27}

To the best of our knowledge, our study is the first to report the association between the levels of *FOXO3a* and the gene polymorphism of *FOXO3a* rs4946936 in the case of CGL patients. Our findings may serve as an initial investigation regarding the role of *FOXO3a* rs4946936 gene polymorphism in affecting the levels of *FOXO3a*, compared to different

findings in the case of COPD and vitiligo. In the future, our results may contribute to a better understanding regarding the role of *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* in the pathogenesis of CGL patients. However, understanding the role of genetics in a disease is problematic and further studies involving gene-gene, gene-environment, and gene-disease interaction are warranted to determine the holistic role of *FOXO3a* gene polymorphism in the pathogenesis of CGL.

Several pertinent limitations to this study should be assessed. First, several factors that might contribute to the severity of CGL in patients including smoking, physical activity, dietary factors, the history of previous medication, and the history of radiation were not analyzed. Second, the small sample size in our study should be interpreted with caution due to the possibility of false positive results. Third, the short time period of the study might not be sufficient to define the real correlation.

Conclusion

Our study indicates that the CC genotype of the *FOXO3a* rs4946936 gene variant is associated with elevated levels of *FOXO3a*. Our findings may be used as an initial investigation and may provide a better understanding of the role of the *FOXO3a* rs4946936 gene variant in the pathogenesis of CGL.

Data availability

Underlying data

Figshare: Underlying data for ‘The association between *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* among chronic granulocytic leukemia patients treated with imatinib mesylate’, <https://doi.org/10.6084/m9.figshare.16529160>.¹⁷

This project contains the following underlying data:

- Data file.xlsx
- Gel electrophoresis.pdf

Reporting guidelines

Figshare: STROBE checklist for ‘The association between *FOXO3a* rs4946936 gene polymorphism and the levels of *FOXO3a* among chronic granulocytic leukemia patients treated with imatinib mesylate’, <https://doi.org/10.6084/m9.figshare.16529160>.¹⁷

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0)

Consent

Written informed consent for publication of the patients’ details was obtained from the patients.

Acknowledgements

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Department of Stem Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

Thank you very much for giving me the opportunity to review the revised manuscript to "F1000Research". I feel that the authors well answered my concern. Thus, I agree to accept this paper to be indexed on "F1000Research".

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 07 April 2022

<https://doi.org/10.5256/f1000research.121907.r125948>

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Kazuhiro Naka 

Department of Stem Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

While the authors improved the description, they have not corrected the introduction yet. The authors described that "FOXO3a plays a dominant role in carcinogenesis including cell cycle

progression, proliferation, the activation of reactive oxygen species, DNA damage, apoptosis, and tumorigenesis." However, it has been known that FOXO3a is implicated in "cell cycle arrest", "detoxification of reactive oxygen species", and "DNA damage repair". Thus, please correct the descriptions.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Tyrosine kinase inhibitor (TKI) resistant relapse of chronic myelogenous leukaemia (CML) patients.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 08 Apr 2022

Jonny Fajar, Universitas Brawijaya, Malang, Indonesia

1. While the authors improved the description, they have not corrected the introduction yet. The authors described that "FOXO3a plays a dominant role in carcinogenesis including cell cycle progression, proliferation, the activation of reactive oxygen species, DNA damage, apoptosis, and tumorigenesis." However, it has been known that FOXO3a is implicated in "cell cycle arrest", "detoxification of reactive oxygen species", and "DNA damage repair". Thus, please correct the descriptions.
Response: We have revised the above description.

Competing Interests: We have no competing interest to declare

Reviewer Report 02 March 2022

<https://doi.org/10.5256/f1000research.121907.r125947>

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Mardiah Hardianti

Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia

I found the responses from the authors were sufficient to answer all our questions. I hereby agree for the work to be indexed without any further reservation.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 10 February 2022

<https://doi.org/10.5256/f1000research.76675.r120814>

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Mardiah Hardianti

Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Dr. Sardjito Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia

1. The idea of this paper is original and highly appreciated. It aimed to elucidate one of the important molecular mechanism of CGL by assessing the target gene polymorphism and its protein FOXO3a. The finding that CC genotype was related to the higher level of FOXO3a which might be related with disease mechanism was explained well by referring to other disease mechanism such as vitiligo and COPD. However, it is still suggested to be explained more in the aspect of molecular mechanism of hematological malignancy.
2. Other possible explanations for the uninvolved role of CT or TT polymorphisms in CGL disease mechanism should be explained more rather than just comparing their biochemical aspect to the CC phenotype which stated that CC phenotype produced higher level of FOXO3a protein. Any technical or sampling issues could also be other explanations for this finding.
3. Is there any possible extrapolation for disease mechanism from Indonesian population regarding CC phenotype findings in this study? Please explain either when the answer is yes or no.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular mechanism of cancer

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Feb 2022

Jonny Fajar, Universitas Brawijaya, Malang, Indonesia

Reviewer2

1. The idea of this paper is original and highly appreciated. It aimed to elucidate one of the important molecular mechanism of CGL by assessing the target gene polymorphism and its protein FOXO3a. The finding that CC genotype was related to the higher level of FOXO3a which might be related with disease mechanism was explained well by referring to other disease mechanism such as vitiligo and COPD. However, it is still suggested to be explained more in the aspect of molecular mechanism of hematological malignancy.

Response: The additional explanation on the possible role of FOXO3a in the context of hematological malignancy has been provided.

2. Other possible explanations for the uninvolved role of CT or TT polymorphisms in CGL disease mechanism should be explained more rather than just comparing their biochemical aspect to the CC phenotype which stated that CC phenotype produced higher level of FOXO3a protein. Any technical or sampling issues could also be other explanations for this finding.

Response: The explanation regarding the possible reason why CC genotype may associate with the higher production of FOXO3a levels compared to CT and TT genotypes has been provided in the discussion paragraph 2 (the possible association between the genotype and free energy levels).

3. Is there any possible extrapolation for disease mechanism from Indonesian population regarding CC phenotype findings in this study? Please explain either when the answer is yes or no.

Response: The additional explanation on the possible gene – environment interaction

including Indonesian population has been provided.

Competing Interests: We have no competing interest to declare.

Reviewer Report 13 January 2022

<https://doi.org/10.5256/f1000research.76675.r118690>

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Kazuhiro Naka 

Department of Stem Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

In the valuable manuscript, the authors investigated association between FOXO3a polymorphism and the levels of FOXO3a in serum from chronic granulocytic leukemia (CGL) patients after treatment with imatinib mesylate. They indicated that the CC, CT, and TT genotypes of the FOXO3a rs4946936 gene polymorphism were 35.0%, 48.3%, and 16.7%, respectively. Interestingly, the levels of FOXO3a were elevated in the CGL patients with CC genotype of the FOXO3a rs4946936 gene than those with the CT and TT genotypes. Collectively, the authors concluded that the CC genotype of the FOXO3a rs4946936 gene polymorphism affects the elevated levels the FOXO3a in CGL patients treated with imatinib mesylate.

Major points

In table 2, it is difficult to understand what it means, while it indicates important findings in this study. Probably, first "Mean" reveals the expression levels of FOXO3a. But, I cannot understand the second "Mean". Please explain each "Mean". I strongly recommend that the authors reveal the data as "bar graphs" rather than table. It helps the understanding of a wide variety of readers.

I understood the authors' findings that the CC genotype of the FOXO3a rs4946936 gene polymorphism appears to be correlated with the expression of FOXO3a in the serum of CGL patients treated with imatinib mesylate. However, the authors should indicate clinical significance of the genotype. For example, they should compare the therapeutic effects of imatinib mesylate between the CC genotype and CT and TT genotypes, such as IS of BCR-ABL1 or deep molecular response (DMR) patients. They should also indicate whether or not the levels of FOXO3a in the CC genotypes are elevated post-imatinib administration than pre-imatinib therapy. These data may contribute to predict the therapeutic effect of imatinib mesylate in the patients.

Minor point

In the introduction, the authors described that "FOXO3a plays a dominant role in carcinogenesis including cell cycle progression, proliferation, the activation of reactive oxygen species, DNA

damage, apoptosis, and tumorigenesis." However, it has been known that FOXO3a is implicated in "cell cycle arrest", "detoxification of reactive oxygen species", and "DNA damage repair". Thus, please correct the descriptions.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Tyrosine kinase inhibitor (TKI) resistant relapse of chronic myelogenous leukaemia (CML) patients.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Feb 2022

Jonny Fajar, Universitas Brawijaya, Malang, Indonesia

Reviewer1

1. In table 2, it is difficult to understand what it means, while it indicates important findings in this study. Probably, first "Mean" reveals the expression levels of FOXO3a. But, I cannot understand the second "Mean". Please explain each "Mean". I strongly recommend that the authors reveal the data as "bar graphs" rather than table. It helps the understanding of a wide variety of readers.

Response: We have provided Figure 2 instead of Table 2, as suggested by reviewer.

2. I understood the authors' findings that the CC genotype of the FOXO3a rs4946936 gene polymorphism appears to be correlated with the expression of FOXO3a in the serum of CGL

patients treated with imatinib mesylate. However, the authors should indicate clinical significance of the genotype. For example, they should compare the therapeutic effects of imatinib mesylate between the CC genotype and CT and TT genotypes, such as IS of BCR-ABL1 or deep molecular response (DMR) patients. They should also indicate whether or not the levels of FOXO3a in the CC genotypes are elevated post-imatinib administration than pre-imatinib therapy. These data may contribute to predict the therapeutic effect of imatinib mesylate in the patients.

Response: In our present study, we did not compare the levels of FOXO3 between pre and post imatinib treatment. Therefore, we could not provide the holistic information regarding those comparison. However, we have added the additional discussion about the proposed effect of FOXO3a levels and the treatment of imatinib.

3. In the introduction, the authors described that "FOXO3a plays a dominant role in carcinogenesis including cell cycle progression, proliferation, the activation of reactive oxygen species, DNA damage, apoptosis, and tumorigenesis." However, it has been known that FOXO3a is implicated in "cell cycle arrest", "detoxification of reactive oxygen species", and "DNA damage repair". Thus, please correct the descriptions.

Response: The above description has been revised.

Competing Interests: We have no competing interest to declare.

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