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GPCR genes as a predictor of glioma severity and clinical outcome

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Abstract

Objective: To undertake a comprehensive analysis of the differential expression of the G protein-coupled receptor (*GPCR*) genes in order to construct a *GPCR* gene signature for human glioma prognosis.

Methods: This current study investigated several glioma transcriptomic datasets and identified the *GPCR* genes potentially associated with glioma severity.

Results: A gene signature comprising 13 *GPCR* genes (nine upregulated and four downregulated genes in high-grade glioma) was developed. The predictive power of the 13-gene signature was tested in two validation cohorts and a strong positive correlation (Spearman's rank correlation test: $\rho = 0.649$ for the Validation1 cohort; $\rho = 0.693$ for the Validation2 cohort) was observed between the glioma grade and 13-gene based severity score in both cohorts. The 13-gene signature was also predictive of glioma prognosis based on Kaplan–Meier survival curve analyses and Cox proportional hazard regression analysis in four cohorts of patients with glioma.

Conclusions: Knowledge of *GPCR* gene expression in glioma may help researchers gain a better understanding of the pathogenesis of high-grade glioma. Further studies are needed to validate the association between these *GPCR* genes and glioma pathogenesis.

Keywords

Gene signature, transcriptome, glioma, *GPCR* genes

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Introduction

Glioma is the most prevalent brain tumour and its diagnosis is based on clinical characteristics, histopathological analysis and radiographic features.[1–4] Conventional tumour grading has not been suitable for outcome prediction in the case of glioma,

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particularly between grades III and IV; in fact, grade IV brain tumours are the most malignant with poor clinical outcomes.[5] The Cancer Genome Atlas provides information on genome alterations in various types of cancers present in large cohorts of humans.[6] Gene expression-based molecular classification has been established with four glioma subtypes: classical, mesenchymal, proneural and neural.[7] To understand the biology of glioma and its optimal treatment, multidisciplinary approaches, including the use of morphological features for imaging-based phenotypic characterization using magnetic resonance images, distant metastasis prediction and molecular characteristic classification, are being used to predict the survival outcome.[8,9] The gene expression analyses based on high-throughput platforms and microarrays have improved the diagnostic ability by incorporating genetic signatures of glioma pathology. For example, genes related to inflammation, energy metabolism, autophagy and DNA methylation have previously been reported as prognostic markers of glioma.[10–14] Furthermore, various genetic alterations, such as mutations in the *IDH1*, *IDH2* and *P53* genes, altered expression of epidermal growth factor receptor and aberrant *MGMT* promoter methylation status, have been reported in gliomas.[15–19]

G-protein-coupled receptors (GPCRs), a family of membrane receptors, play an important role in cellular signalling involving ligands, hormones, neurotransmitters and proteases.[20] Indeed, GPCRs relevant to tumour growth and metastasis have been observed in many forms of cancer.[21–24] Numerous GPCRs bearing ligand-binding sites are located on the extracellular domain with agonist, thereby, these sites are easily accessible at the cell surface membrane.[25] Therefore, GPCRs have become the focus of research interest as novel drug targets for several diseases.[26–28]

This current study undertook a comprehensive analysis of the differential expression of the *GPCR* genes in order to construct a *GPCR* gene signature for human glioma prognosis.

Materials and methods

GPCR genes

The definition of *GPCR* genes was obtained from the IUPHAR database (https://www.guidetopharmacology.org/).[29] Only the well-annotated human *GPCR* genes were considered. A total of 407 *GPCR* genes were collected in this study (see supplementary materials, Table S1), including 5-hydroxytryptamine receptors, acetylcholine receptors, adenosine receptors, adhesion class GPCRs, adrenoceptors, angiotensin receptors, apelin receptor, bile acid receptor, bombesin receptors, bradykinin receptors, calcitonin receptors, calcium-sensing receptor, cannabinoid receptors, chemerin receptors, chemokine receptors, cholecystokinin receptors, class A orphans, class C orphans, class frizzled GPCRs, complement peptide receptors, corticotropin-releasing factor receptors, dopamine receptors, endothelin receptors, formylpeptide receptors, free fatty acid receptors, G protein-coupled oestrogen receptor, gamma-aminobutyric acid (GABA) B receptors, galanin receptors, ghrelin receptor, glucagon receptor family, glycoprotein hormone receptors, gonadotrophin-releasing hormone receptors, histamine receptors, hydroxycarboxylic acid receptors, kisspeptin receptor, leukotriene receptors, lysophospholipid, receptors, melanin-concentrating hormone receptors, melanocortin receptors, melatonin receptors, metabotropic glutamate receptors, motilin receptor, neuromedin U receptors, neuropeptide FF/neuropeptide AF receptors, neuropeptide S receptor, neuropeptide W/neuropeptide B receptors, neuropeptide Y receptors,

neurotensin receptors, opioid receptors, opsin receptors, orexin receptors, oxoglutarate receptor, purinergic receptors, parathyroid hormone receptors, platelet-activating factor receptor, prokineticin receptors, prolactin-releasing peptide receptor, prostanoid receptors, proteinase-activated receptors, pyroglutamylated RFamide peptide receptor, relaxin family peptide receptors, somatostatin receptors, succinate receptor, tachykinin receptors, taste 1 receptors, taste 2 receptors, thyrotropin-releasing hormone receptors, trace amine receptor, urotensin receptor, vasopressin and oxytocin receptors, vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors.

Glioma transcriptomic data

Seven glioma transcriptomic datasets were investigated in this study, which were generated using the following arrays: Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix Human Genome U133A/B Array, Affymetrix Human Genome U95Av2 Array or Affymetrix Human Exon 1.0 ST Array. The Robust Multi-array Average (RMA) tool was used to quantify the genome-wide gene expression profile for the Affymetrix Human Genome U133 Plus 2.0 Array, U133A/B Array and U95Av2 Array;[30] while the Affymetrix Human Exon 1.0 ST Array data were summarized using Affymetrix Power Tools. One of the transcriptomic datasets was used as a discovery cohort and all the other datasets were used for validation purposes. In the discovery cohort, only the probesets with unique annotations that were present in at least two-thirds of the samples were retained for further analysis.

Severity score based on GPCR gene expression

A signature based on multiple GPCR genes was developed, which can be used to predict severity and survival for glioma patients. A severity score for each glioma patient was computed based on the gene expression information of the signature, which was a combination of the expression values of the *GPCR* genes within the signature:[31]

$$S = \sum_{i=1}^{n} W_i(e_i - \mu_i)/\tau_i$$

Here, S was the severity score; n was the number of genes in the signature; W_i denoted the weight of gene i in the signature (+1 was assigned to the genes that were upregulated in high-grade glioma while –1 was assigned to the genes that were downregulated in high-grade glioma); e_i was the expression level of gene i; μ_i was the mean of the expression for gene i across the samples in each cohort; and τ_i was the standard deviation of the expression for gene i across the samples in each cohort.[31]

Statistical analyses

All statistical analyses were undertaken using the R statistical package (R version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria). Spearman's rank correlation test was performed using the 'cor.test' function. The 'pROC' package was used to generate the receiver operating characteristic (ROC) curve and compute the area under the ROC curve (AUC). The 'survival' package was used to perform the log-rank test using the 'survdiff' function and Cox proportional hazard regression using the 'coxph' function. A P-value <0.05 was considered statistically significant.

Results

First, the *GPCR* genes that were correlated with glioma World Health Organization grade were identified. A glioma

transcriptomic dataset was obtained from the Gene Expression Omnibus (GEO) database with an accession of GSE43289,[32] which was used as a discovery cohort to prioritize the *GPCR* genes that are associated with glioma severity. In total, the *GPCR* genes in three grade I patients, three grade II patients, six grade III patients and 28 grade IV patients were investigated. The correlation between *GPCR* gene expression and glioma grade was computed by Spearman's rank correlation test. Figure 1 presents the *GPCR* genes with a correlation coefficient $\rho > 0.2$ or $<$–0.2, which represents a list of *GPCR* genes that are potentially differentially expressed in glioma: a positive P suggests upregulation while a negative P suggests downregulation in high-grade glioma. Among these genes, the genes with an adjusted P-value $<$0.05 were selected. This step yielded a list of 13 *GPCR* genes (Figures 1 and 2), which included nine genes upregulated in high-grade glioma (*CELSR1*, *F2RL2*, *FZD1*, *FZD2*, *FZD5*, *FZD7*, *GPR107*, *HRH1* and *P2RY1*) and four genes downregulated in high-grade glioma (*GABBR1*, *GPRC5B*, *HTR2A* and *P2RY12*). These 13 *GPCR* genes were assigned as a 13-gene signature. To test whether the correlation between *GPCR* gene expression and glioma grade was robust, two independent validation cohorts were investigated: Validation1 and Validation2. The Validation1 cohort (GEO accession number: GSE4290)[33] consists of 23 non-glioma subjects and 45 grade II, 31 grade III, and 77 grade IV glioma patients; while the Validation2 cohort (GEO accession number: GSE19728)[34] consists of four non-glioma subjects, and two grade I, five grade II, five grade III and five grade IV glioma patients. Spearman's rank correlation test between expression and grade was applied to the *GPCR* genes listed in Figure 1. The ρ of the discovery cohort was significantly correlated with the ρ of the two validation cohorts (Spearman's rank correlation test: $\rho = 0.859$ and $P < 10^{-10}$ for the Validation1 cohort; $\rho = 0.640$ and $P = 2.2 \times 10^{-6}$ for the Validation2 cohort) (Figure 3), which suggests that the *GPCR* gene expression profile in the discovery cohort can be robustly

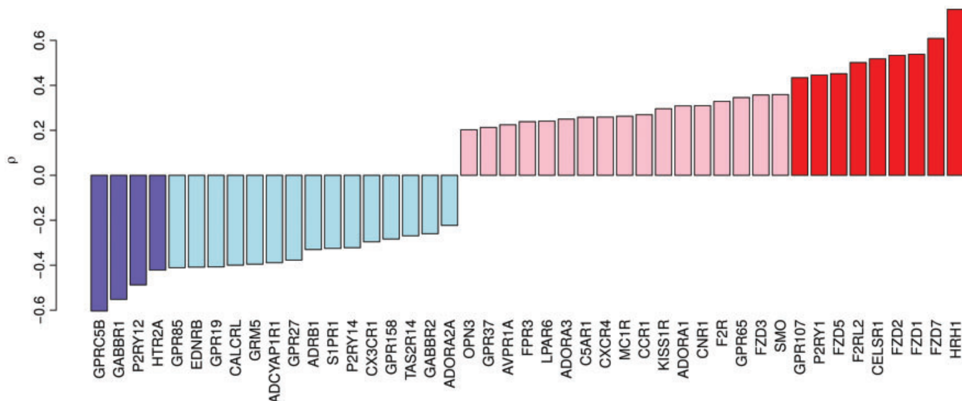

Figure 1. The G-protein-coupled receptor (*GPCR*) genes potentially correlated with glioma severity. The correlation coefficient between *GPCR* gene expression and glioma grade was calculated using Spearman's rank correlation test. Only the *GPCR* genes with $\rho > 0.2$ were retained. A positive ρ suggests upregulation in high-grade glioma while a negative ρ suggests downregulation in high-grade glioma. The colour version of this figure is available at: http://imr.sagepub.com.

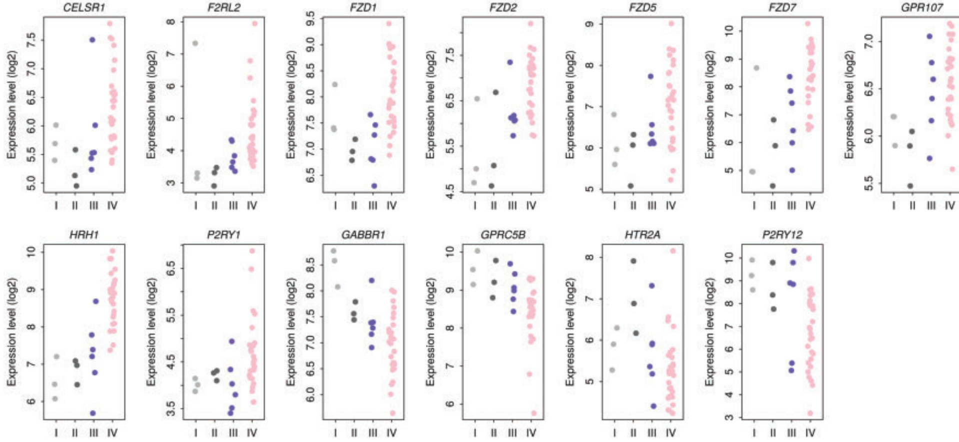

Figure 2. The 13-gene signature. The G-protein-coupled receptor (*GPCR*) gene expression data were derived from the discovery cohort. For the *GPCR* genes with multiple probesets, only the probeset with the most significant *P*-value was demonstrated. The X-axis denotes glioma grade while the Y-axis represents $\log_2$-transformed gene expression. The colour version of this figure is available at: http://imr.sagepub.com.

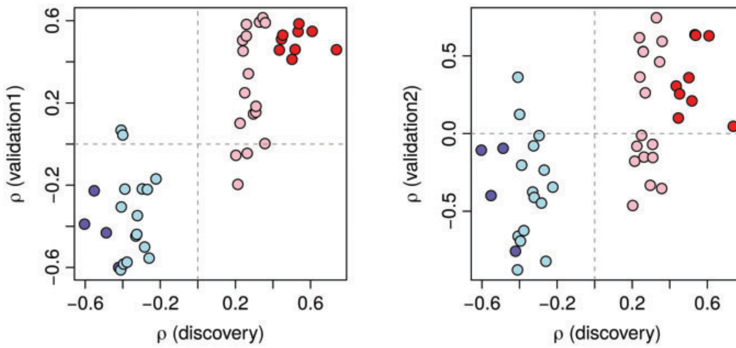

Figure 3. The ρ between G-protein-coupled receptor (*GPCR*) gene expression and glioma grade. Each dot denotes a *GPCR* gene. Only the genes listed in Figure 1 were included. The pink and red dots stand for the genes with positive ρ in the discovery cohort, while the dark and light blue dots represent the gene with negative ρ in the discovery cohort. The left and right panels are for the Validation1 and Validation2 cohorts, respectively. The ρ in the discovery cohort is significantly correlated with that of the two validation cohorts. The colour version of this figure is available at: http://imr.sagepub.com.

mirrored by the pattern in the two validation cohorts.

A 13-gene signature based on 13 *GPCR* genes with nine upregulated and four downregulated in high-grade glioma was developed. It was assumed that the expression of the 13-gene signature could be used to predict glioma severity, i.e. tumour grade. A weight was assigned to each gene among the 13-gene signature according to the direction of differential expression, i.e. +1 for the upregulated genes and –1 for the downregulated genes in high-grade glioma. In the discovery cohort, a severity score was assigned to each subject based on the expression pattern of the 13-gene signature

as detailed in the 'Materials and methods' section. A greater severity score suggests more severe glioma. A strong positive correlation (Spearman's rank correlation test: $\rho = 0.739$ and $P = 5.2 \times 10^{-8}$) was observed between the tumour grade and 13-gene based severity score (Figure 4a). The predictive power of the 13-gene signature was tested in the two validation cohorts. In both validation cohorts, a strong positive correlation (Spearman's rank correlation test: $\rho = 0.649$ and $P < 10^{-10}$ for the Validation1 cohort; $\rho = 0.693$ and 2.2×10^{-4} for the Validation2 cohort) was observed between the glioma grade and 13-gene based severity score (Figure 4a), which suggests a promising predictive power of the 13-gene signature in predicting glioma severity. In addition, the ROC curve was plotted to quantify the classification power of the 13-gene based severity score in differentiating between the non-glioma and glioma subjects (Figure 4b). The AUC was 0.844 between the glioma and non-glioma subjects in the Validation1 cohort. All these results suggest that the 13-gene signature can be used not only for glioma severity prediction but also for glioma screening.

The study then determined whether the 13-gene based severity score could be used to predict clinical outcome in glioma by investigating four independent glioma transcriptomic datasets: (i) the European Organisation for Research and Treatment of Cancer (EORTC) cohort including 95 glioma patients with a GEO accession of GSE43107;[35] (ii) the University of California at Los Angeles (UCLA) cohort including 85 glioma patients with a GEO accession of GSE4412;[36] (iii) the Massachusetts General Hospital (MGH) cohort composed of 50 glioma patients;[37] and (iv) the MD Anderson Cancer Center (MDACC) cohort including 77 glioma patients with a GEO accession of GSE4271.[38] The severity score was computed for all the subjects within the four validation cohorts based on the GPCR gene expression of the 13-gene signature. Kaplan–Meier survival curves demonstrated a significant difference in survival between the glioma patients with positive and negative severity scores in the four cohorts (log-rank test: $P = 2.0 \times 10^{-3}$ for the EORTC cohort; $P = 3.0 \times 10^{-3}$ for the UCLA cohort; $P = 2.0 \times 10^{-4}$ for the MGH

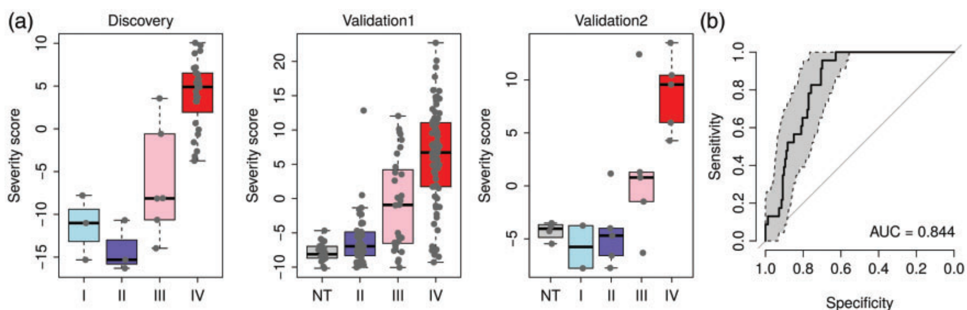

Figure 4. The performance of the 13-gene signature in predicting glioma severity: (a) comparison of the 13-gene based severity score in different categories. The heavy central black horizontal lines for each grade are the median; the extremities of the boxes are the 25th and 75th percentiles; and the whiskers represent 1.5 interquartile ranges (IQRs) (in case there is no data point exceeding 1.5 IQR, the lower/upper whiskers denote the minimum/maximum values respectively). NT, non-tumour samples and (b) The receiver operating characteristic curve of the 13-gene based severity score in distinguishing between the glioma and non-glioma subjects in the Validation1 cohort. The colour version of this figure is available at: http://imr.sagepub.com.

cohort; $P = 3.0 \times 10^{-4}$ for the MDACC cohort) (Figure 5). The relationship between the severity score and survival was further confirmed by Cox proportional hazard regression analysis. Compared with the patients with a negative severity score, the patients with a positive severity score had a 2.84-fold increased risk of death in the EORTC cohort ($P = 2.8 \times 10^{-3}$), 2.51-fold in the UCLA cohort ($P = 3.4 \times 10^{-3}$), 2.51-fold in the MGH cohort ($P = 4.7 \times 10^{-4}$) and 3.95-fold in the MDACC cohort ($P = 4.2 \times 10^{-4}$). All these results suggest that the 13-gene signature can be used for glioma prognosis.

Discussion

To date, various anatomical and histological features and molecular alterations related to glioma have been identified, enabling the development of various novel approaches for the identification of glioma progression.[9,39–42] Although several molecular signatures have been used to predict glioma, they are yet to be adopted clinically for the prognostic evaluation of glioma patients.[43] Diverse and precise molecular profiling resources will be needed to provide diagnosis and prognosis predictions of glioma. GPCRs are critically involved in cell proliferation and survival and are abnormally expressed in tumour cells.[44] With increasing evidence that demonstrates the crucial role of GPCRs in carcinogenesis, this current study aimed to develop prognostic molecular signatures based on the expression of aberrant *GPCR* genes to predict the survival of patients with glioma. In this current study, the *GPCR* genes that were differentially expressed in glioma patients were identified and a 13-gene signature was established to improve the prognostic evaluation of glioma. The grade-dependent *GPCR* genes were significantly associated with survivability in glioma cohorts.

The frizzled (FZD) family of proteins consists of 10 isoforms (FZD1–10) and many aspects of FZD signal transduction are critical for cell cycle regulation, embryonic development, cell proliferation and the development of the central nervous system.[45–49] Accumulating evidence shows that aberrant FZD signalling is involved in many different types of cancers and a critical role of FZD has been reported in the later stages of tumour development.[50–52] For example, the expression of FZD2 and FZD7 was upregulated in later-stage tumours in pancreatic adenocarcinoma; and FZE10 was highly expressed in the later stages of tumour progression in colon cancer.[53,54] In particular, FZD7

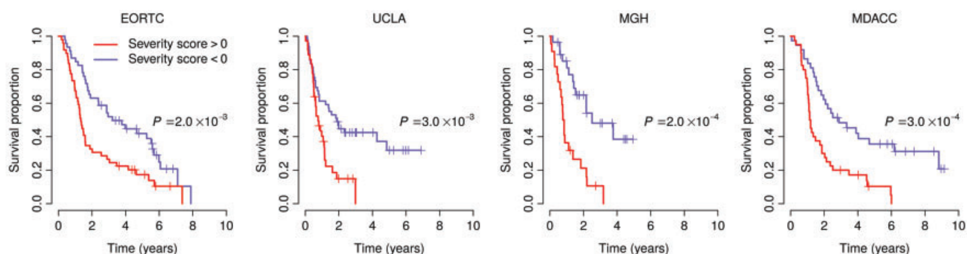

Figure 5. Kaplan-Meier curves for the EORTC, UCLA, MGH and MDACC cohorts. The 13-gene based severity score predicts clinical outcome in all the cohorts. *P*-values were calculated using log-rank test. EORTC, European Organisation for Research and Treatment of Cancer; UCLA, University of California at Los Angeles; MGH cohort, Massachusetts General Hospital; MDACC, MD Anderson Cancer Center. The colour version of this figure is available at: http://imr.sagepub.com.

upregulation in glioma patients was associated with a poor prognosis.[55] In fact, aberrant FZD signalling and complex formation between FZDs and Wnt have been associated with several human cancers.[56] Blocking FZDs using antibodies or inhibitors has been explored as an anticancer therapy and is known to disrupt Wnt signalling and inhibit their downstream genes.[51,57] In this current study, higher expression levels of FZD1, FZD2, FZD5 and FZD7 were identified in glioma tissues than in normal tissues. In addition to the FZD family, prominent expression of purinergic receptors P2RY1 was identified in high-grade glioma. Previous studies on prostate and gastric cancers have demonstrated that extracellular adenosine triphosphate promotes cancer progression through the activation of purinergic receptors and the resulting induction of apoptosis.[58–60] This current study also identified increased expression of *HRH1* in higher grades of glioma.

In this current study, a low expression of *GPCR* genes, such as *GABBR1*, *GPRC5B*, *HTR2A* and *P2RY12* was observed in high-grade glioma. GABA is an inhibitory neurotransmitter in the nervous system, which functions via both $GABA_A$ and $GABA_B$ receptors. Considering the importance of $GABA_B$ receptors in maintaining neuronal excitability, the lack of the $GABA_{B1}$ subunit causes epileptic seizures, pain disorders and an impaired memory.[61] GPRC5B is an orphan receptor belonging to the GPCR family and contributes to neurogenesis.[62] The 5-hydroxytryptamine receptor *HTR2A* gene is a prognostic marker for low-grade glioma[63] and downregulation of HTR5A has been found in high-grade glioma.[64]

This current study identified 13 *GPCR* genes that can be used to predict the severity and clinical outcomes of glioma. As this gene signature was derived from grade-specific *GPCR* genes and strongly associated with glioma severity, it is reasonable to anticipate that this gene signature can be used to monitor glioma treatment. A change in the 13-gene based severity score potentially reflects the treatment response in glioma patients, which may be applicable for optimizing the treatment plan for individual patients. Further studies are needed to validate the association between these *GPCR* genes and glioma pathogenesis for a better clinical application.

Author contributions

E.K. and T.Z. conceived the idea. T.Z. performed the statistical analysis. E.K. interpreted and discussed the results. E.K. and T.Z. wrote the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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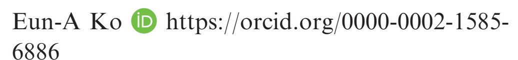
Eun-A Ko  https://orcid.org/0000-0002-1585-6886

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