DOI:10.34084/bshr.1719676 e-ISSN 2587-1641

Journal of Biotechnology and Strategic Health Research

Araştırma Makalesi /Research Article

http://dergipark.org.tr/tr/pub/bshr

Expression Profiling of Circular RNAs in *Staphylococcus aureus*-Infected Macrophages

Staphylococcus aureus ile Enfekte Makrofajlarda Halkasal RNA'ların Ekspresyon Profili

Ufuk Mert[1,2,3], Deniz Ece[1], Kerem Güngör[4], Tolga Coşkun[1], Uygar Alaman[1], Gökhan Gurur Gökmen[5], Ayşe Caner[1,3,6]

[1] Ege University, Department of Basic Oncology, Institute of Health Sciences, Izmir, Türkiye

[2] Ege University, Atatürk Vocational School of Health Services, Izmir, Türkiye

[3] Ege University, Translational Pulmonary Research Center (EGESAM), Izmir, Türkiye

[4] İzmir Institute of Technology, Department of Molecular Biology and Genetics, Faculty of Science, Izmir, Türkiye

[5] Alanya University, Vocational School Department of Food Processing, Food Quality Control and Analysis Program, Antalya, Turkiye

[6] Ege University, Department of Parasitology, Faculty of Medicine, Izmir, Türkiye

ORCID ID: Ufuk Mert: https://orcid.org/0000-0001-8162-5325, Deniz Ece: https://orcid.org/0009-0000-2787-2640
Kerem Güngör: https://orcid.org/0009-0001-3911-4668, Tolga Coşkun: https://orcid.org/0009-0007-9263-6549
Uygar Alaman: https://orcid.org/0009-0009-4932-5010, Gökhan Gurur Gökmen: https://orcid.org/0000-0002-1180-0682
Ayşe Caner: https://orcid.org/0000-0003-3058-9971

***Sorumlu Yazar / Corresponding Author:** Ayşe Caner, **e-posta / e-mail:** : ayse.caner@ege.edu.tr

Geliş Tarihi / Received : 14-06-2025 **Kabul Tarihi / Accepted:** 01-07-2025 **Yayın Tarihi / Online Published:** 31-08-2025

Mert U., Ece D., Güngör K. et al. Staphylococcus aureus ile enfekte makrofajlarda halkasal RNA'ların Ekspresyon Profili, J Biotechnol and Strategic Health Res. 2025;9(2):114-123

Abstract

Aim Understanding host-pathogen interactions and identifying novel targets are crucial for controlling Staphylococcus aureus (S. aureus) infections. Circular RNAs (circRNAs), a class of non-coding RNAs, have gained attention due to their stable structure, circular configuration, and roles in biological mechanisms. They are detectable in bodily fluids and tissues, suggesting potential as biomarkers. This study aimed to investigate the circRNA expression profile in S. aureus-infected macrophages and explore their potential role in host-pathogen interactions.

Materials and Methods THP-1 human monocytic cells were differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA). THP-1-derived macrophages were infected with S. aureus ATCC 6538P at ratios of 1:25, 1:100, and 1:250. Extracellular bacteria were eliminated using gentamicin. RNA was isolated with Trizol reagent, and cDNA was synthesized. The expression levels of 15 selected circRNA genes were analyzed via RT-qPCR. Gene Ontology (GO) analyses were performed to assess their roles in molecular biological processes.

Results THP-1 cells were successfully differentiated into macrophages, establishing an infection model. As the infection ratio increased, total RNA concentrations decreased. RT-qPCR revealed that 11 circRNAs were differentially expressed in at least one infection group. Notably, hsa_circ_0001821 was downregulated at a 1:25 infection ratio ($p<0.01$), hsa_circ_000869 was significantly upregulated at 1:250 ($p<0.0001$), and hsa_circ_0014130 was downregulated at 1:250 ($p<0.0001$). GO analyses indicated that infection-related circRNAs regulate RNA/mRNA catabolic processes and cell proliferation.

Conclusions This study demonstrates that S. aureus infection alters macrophage circRNA expression in a dose-dependent manner. These findings highlight circRNAs as potential regulators of host-pathogen interactions, providing a foundation for novel diagnostic and therapeutic strategies.

Keywords Infection, Macrophage, Circular RNA, THP-1, Staphylococcus aureus

Öz

Amaç *Staphylococcus aureus (S. aureus) enfeksiyonun kontrolü için konakçı-patojen etkileşimlerinin anlaşılması ve yeni hedeflerin tanımlanması gerekmektedir. Son yıllarda kodlamayan RNA sınıfından olan halkasal RNA'lar (circRNA), stabil yapıları ve dairesel konfigürasyonları ile biyolojik mekanizmalarda önemli rol oynamakta, vücut sıvılarında ve dokularında bulunmaktadır. Bu çalışma, S. aureus ile enfekte edilmiş makrofajlarda circRNA ekspresyon profilini incelemeyi ve konakçı-patojen etkileşimlerinde circRNA'ların potansiyel rolünü araştırmayı amaçlamıştır.*

Gereç ve Yöntem *Makrofaj modeli oluşturmak için THP-1 insan monositik hücreleri kullanılmıştır. THP-1 hücreleri, Phorbol 12-myristate 13-acetate (PMA) ile makrofajlara farklılaştırılmıştır. Daha sonra, THP-1 türevi makrofajlar, S. aureus ATCC 6538P suşu ile 1:25, 1:100 ve 1:250 oranlarında enfekte edilmiştir. Enfeksiyon sonrası hücre dışındaki bakterileri elimine etmek için gentamisin uygulanmıştır. Makrofajlardan RNA izolasyonu Trizol reaktifi kullanılarak yapılmış ve cDNA sentezlenmiştir. Belirlenen 15 adet circRNA geninin ekspresyon seviyeleri RT-qPCR ile analiz edilmiştir. Gen Ontolojisi (GO) analizleri ile circRNA'ların moleküler biyolojik süreçlerdeki rolleri değerlendirilmiştir*

Bulgular *THP-1 hücreleri makrofajlara başarılı bir şekilde farklılaştırılmış ve makrofaj-enfeksiyon modeli oluşturulmuştur. Enfeksiyon oranı arttıkça total RNA konsantrasyonlarında azalma tespit edilmiştir. RT-qPCR sonuçlarına göre 11 farklı circRNA geninin deney gruplarının en az birinde ifade edildiği belirlenmiştir. Özellikle, hsa_circ_0001821 geninin 1:25 enfeksiyon oranında azaldığı ($p<0.01$), hsa_circ_000869 ifadesinin 1:250 enfeksiyon oranında belirgin bir şekilde arttığı ($p<0.0001$) ve hsa_circ_0014130 ifadesinin 1:250 enfeksiyon durumunda azaldığı ($p<0.0001$) tespit edilmiştir. GO analizleri, enfeksiyon ile ilgili circRNA'ların RNA/mRNA-hücresel katabolik süreçlerin düzenlenmesinde ve hücre proliferasyonunda rolleri olduğu gösterilmiştir.*

Sonuç *Bu çalışma, S. aureus enfeksiyonuna maruz kalan makrofajlarda circRNA ekspresyon profillerinin doza bağımlı olarak değişebileceğini ortaya koymuştur. Özellikle circRNA'ların konakçı-patojen etkileşimlerinin anlaşılması ve yeni tanı/tedavi stratejilerinin geliştirilmesi için önemli bir temel oluşturmaktadır.*

Anahtar Kelimeler *Enfeksiyon, Makrofaj, halkasal RNA, THP-1, Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus (S. aureus) is a gram-positive bacterium capable of causing a wide range of infections in humans, from skin and soft tissue infections to life-threatening systemic diseases such as sepsis and endocarditis[1]. The pathogenicity of *S. aureus* is largely attributed to its virulence factors, including a variety of toxins and enzymes that contribute to host cell damage and the disruption of immune responses[2]. Moreover, *S. aureus* is a major cause of healthcare-associated infections and has demonstrated a remarkable ability to develop resistance to many antimicrobial agents[3]. Consequently, *S. aureus* is recognized as a significant pathogen in both community and clinical settings, and the urgent need for new therapeutic strategies remains a major public health concern. A deeper understanding of host-pathogen interactions and bacterial adaptations within the host immune system may reveal novel targets for infection control and support the development of innovative therapeutics.

Macrophages play a critical role in controlling bacterial burden and limiting mortality associated with S. aureus infections[4]. However, *S. aureus* has evolved sophisticated mechanisms to evade or manipulate macrophage-mediated immune responses to its advantage[5]. It can survive and replicate within macrophages, particularly inside phagosomes[4,6], inhibit host cell death mechanisms[7], and adopt phenotypes associated with increased antibiotic tolerance8. Therefore, elucidating the molecular mechanisms and identifying gene targets triggered by intracellular *S. aureus* is crucial for the development of effective preventive and therapeutic interventions. Previous studies have shown that *S. aureus* infection significantly alters the expression of inflammatory cytokine genes involved in the chemotaxis of host monocytes/macrophages, suggesting their pivotal role in the immune response to infection[9].

Circular RNAs (circRNAs) are a newly identified class of non-coding RNAs characterized by a covalently closed circular structure, which enhances their resistance to exonuclease degradation. CircRNAs exhibit high stability and a notable degree of tissue specificity, often associated with specific developmental stages of tissues and organs[10,11]. Present in various body fluids and tissues, circRNAs perform diverse functions, including regulation of gene transcription and splicing, acting as miRNA sponges, and serving as scaffolds for protein complexes[10]. These properties, combined with their presence in biofluids, render circRNAs promising candidates as clinical biomarkers and therapeutic targets in various diseases[12,13]. Recent studies have highlighted the roles of circRNAs in host cell responses to pathogens[14]. Notably, circRNA_051239, circRNA_029965, and circRNA_404022 have been proposed as potential diagnostic biomarkers for infections caused by Mycobacterium tuberculosis[15].

In this study, an infection model was established to investigate the effects of *S. aureus* on macrophages at the gene expression level. CircRNA genes significantly expressed in macrophages in response to infection were identified, aiming to elucidate their roles in host-pathogen interactions and their potential as novel therapeutic or diagnostic targets.

MATERIALS and METHODS

THP-1 Cell Culture and Differentiation into Macrophages
To establish a macrophage-infection model, suspended human monocytic THP-1 cells were used. THP-1 cells were kindly provided by Prof. Dr. XXXX from the Department of Bioengineering, XXX University. The cells were cultured in RPMI-1640 medium (Sigma), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Life Technologies), 1% (v/v) penicillin/streptomycin, and GlutaMax (Gibco), in a humidified incubator at 37 °C with 5% CO_2.

THP-1 monocytes were seeded in 9.6 cm^2 flat-bottomed plates at a density of 500,000 cells per well. Subsequently, Phorbol 12-myristate 13-acetate (PMA) was added at a final concentration of 250 ng/mL per well. After 24 hours,

the medium was removed, and cells were washed three times with phosphate-buffered saline (PBS). Differentiation of THP-1 monocytes into macrophages following PMA stimulation was confirmed by microscopic observation of changes in cell morphology and adherence properties16.

Staphylococcus aureus Culture

The *Staphylococcus aureus* ATCC 6538P strain was used in this study. For activation, 1 mL of *S. aureus* stored at −20 °C in 25% glycerol was inoculated into 9 mL of Tryptic Soy Broth (TSB; pH 7.3 ± 0.2, Oxoid, UK) and incubated at 37 °C for 24 hours. On the following day, 1 mL from the overnight culture was transferred into fresh 9 mL TSB for secondary activation. The bacterial suspension was incubated at 37 °C until an optical density (OD_{600}) of 0.6 was reached.

Infection of THP-1 Derived Macrophages with *S. aureus*

THP-1-derived macrophages were infected with *S. aureus*. Sterile glass coverslips were placed into the wells for each experimental group to monitor morphological changes and infection status. THP-1 monocytes seeded on the coverslips were differentiated into macrophages as previously described.

For infection, 10 mL of twice-activated bacterial culture was centrifuged at 3000×g for 5 minutes. The supernatant was discarded, and the bacterial pellet was washed twice with PBS, then resuspended in 3 mL of RPMI-1640 medium supplemented with 5% FBS. The bacterial concentration was determined by measuring the OD_{600} using a NanoSpectrophotometer N60 (Implen, Germany), and the number of bacteria was calculated accordingly.

Macrophages were infected with S. aureus at multiplicities of infection (MOI) of 1:25, 1:100, and 1:250 (macrophage: bacteria ratio) and incubated at 37 °C for 2 hours16. After infection, extracellular bacteria were eliminated by treating the cells with gentamicin (100 µg/mL) for 2 hours17. Cells were then washed three times with PBS and collected in the presence of Trizol reagent (tripleXtractor, Grisp, Portugal). Morphological changes were observed under a light microscope (CX23, Olympus, Germany). The coverslips were fixed with methanol and stained with 1:20 diluted Giemsa for 10 minutes. Morphological alterations and infection rates were visualized at 100x magnification under a light microscope.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from infected and non-infected THP-1-derived macrophages using Trizol reagent according to the manufacturer's instructions. RNA concentration and purity were measured by a NanoSpectrophotometer N60 (Thermo Scientific, USA) using A260/280 and A260/230 absorbance ratios. Complementary DNA (cDNA) for circRNAs was synthesized using a cDNA synthesis kit (Nepenthe, Turkey) following the manufacturer's protocol. All RNA samples were stored at −80 °C, while cDNA samples were stored at −20 °C.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

RT-qPCR analyses were performed using the LightCycler 480 system (Roche, USA). The expression of circRNA genes was analyzed in 10 µL reaction volumes using SYBR Green I Master Mix (Roche, USA). Each reaction contained 2 µL cDNA, 5 µL 2x SYBR Green I Master Mix, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), and 1 µL nuclease-free water. The thermal cycling conditions were: 95 °C for 10 s (denaturation), 60 °C for 10 s (annealing), and 72 °C for 10 s (extension). Specificity of the PCR amplification products was confirmed by electrophoresis on 2% agarose gels. β-Actin was used as an internal reference gene, and results were normalized to its expression levels. The primer sequences for β-Actin were: forward: 5'-ATGATGATATCGCCGCGCTC-3', reverse: 5'-TCGTCGCCCACATAGGAATC-3'

Primer Design and Pathway Analysis

CircRNAs to be analyzed in infected cells were selected from the literature using the keywords "circRNA", "apoptosis", "cell proliferation", and "infection", resulting in 15 candidate circRNAs. Divergent primers specific to these circRNAs were designed using the Circular RNA Interactome online tool (https://circinteractome.nia.nih.gov/index.html). All primer sequences are provided in Table 1. The expression levels of circRNAs in each experimental group were assessed using RT-qPCR. Each experiment was performed in triplicate. Gene expression levels of circRNAs were calculated using the ΔΔCt method.

Gene ontology (GO) analysis was conducted using the online platform https://awi.cuhk.edu.cn. CircRNA IDs and related gene information were obtained from the CircBank database (http://www.circbank.cn/).

Statistical Analysis

Differences between control and infected groups were analyzed using GraphPad Prism software (GraphPad Software Inc., CA, USA). The normality of data distribution was assessed before comparing gene expression levels across the four experimental groups. If parametric assumptions were met, one-way ANOVA was applied followed by Tukey's HSD test for post-hoc comparisons. For non-parametric data, the Kruskal–Wallis test was used, and significant differences were further analyzed with Dunn's test. A p-value less than 0.05 was considered statistically significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ***$p < 0.0001$).

RESULTS

The infection of THP-1 cells with the *S. aureus* ATCC 6538P strain was successfully established. In this infection model, THP-1 cells were first differentiated into M0 macrophages. The macrophage morphology was examined under a light microscope16,18. Microscopic analysis revealed that THP-1 monocytes displayed a rounded shape and a non-adherent (suspended) pattern, whereas THP-1-derived macrophages exhibited a typical flat, amoeboid, elongated, and branched morphology, indicative of adherent macrophage differentiation (Figure 1).

Table 1. Sequences and Primer Information of the circRNAs

CircRNA ID	Forward Primer	Reverse Primer
hsa circ_0025036	TGGTCCAGAAGGAGACCTTG	GCCAACCGCTACTTGACATT
hsa_circ_0014130	AAAGTCCGAGGGTTCTGGTT	TGGTACATGACGGAGACACTG
hsa_circ_0002483	TGTGATTCAAGTTGGGGTCA	CGTTATTTGCCAAAAGGATTTC
hsa_circ_0008717	GGTGGCCAGATTAGGTGAGA	CATTCCGTCAGGATCTGTCA
hsa_circ_0043256	TCGAAAGTCACCCCGAATAG	AGTGACGGTGGACTGCTCTT
hsa_circ_0001821	TTGGGTCTCCCTATGGAATG	CATCTTGAGGGGCATCTTTT
hsa_circ_0000285	TACCTCTGCAGGCAGGAACT	TCACATGAATTTAGGTGGGACTT
hsa_circ_0000523	ACATGCTGCCTCAGATGTTG	ACGGAAGGTTGGAGACAATG
hsa_circ_0005320	CCTGTGGCTGAGGCTACAC	ACAGTGGCTCGGAGTAGGG
hsa_circ_0000869	CTCCCAAAGTGCTGGGATTA	ACCCAGCAGGTCATCAAAAC
hsa_circ_0008603	CGGTCTCCTGTTGCTCACTT	GCAGTAACCCATTCAGGAAGA
hsa_circ_0005035	GATGAGCAGGATGTGGAGGT	AGCCGATGTGTGAGAAGACC
hsa_circ_0008042	CTATCAGTGCCCGCTTTGTT	GGCCACTATGAAGAGGCTGA
hsa_circ_0032822	CAAGGAATCTGAGTTGCAGTG	CTTCTCCAGCTGACCACGAT
hsa_circ_0004771	TCCGGATGACATCAGAGCTA	TGTGCATCTTCTGGCTGTGT

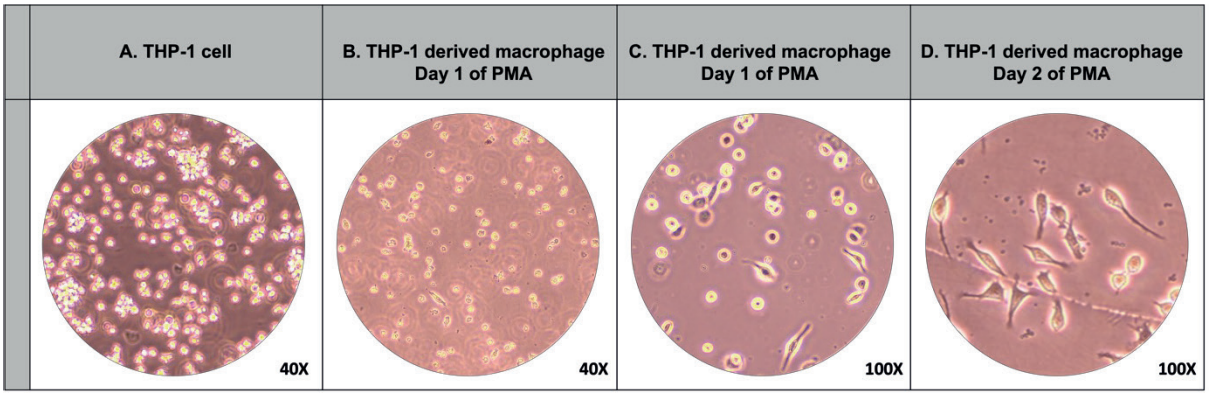

Figure 1. Differentiation of THP-1 monocytes into macrophages. A. Undifferentiated THP-1 monocytes, B. PMA-stimulated THP-1-derived macrophages on day 1 (magnification 40x), C. PMA-stimulated THP-1-derived macrophages on day 1 (magnification 100x), D. PMA-stimulated THP-1-derived macrophages on day 2 (magnification 100x).

Following macrophage differentiation, cells were infected with S. aureus at multiplicity of infection (MOI) ratios of 1:25, 1:100, and 1:250. At the 4th hour post-infection, the coverslips were stained with Giemsa to demonstrate morphological changes and infection status (Figure 2).

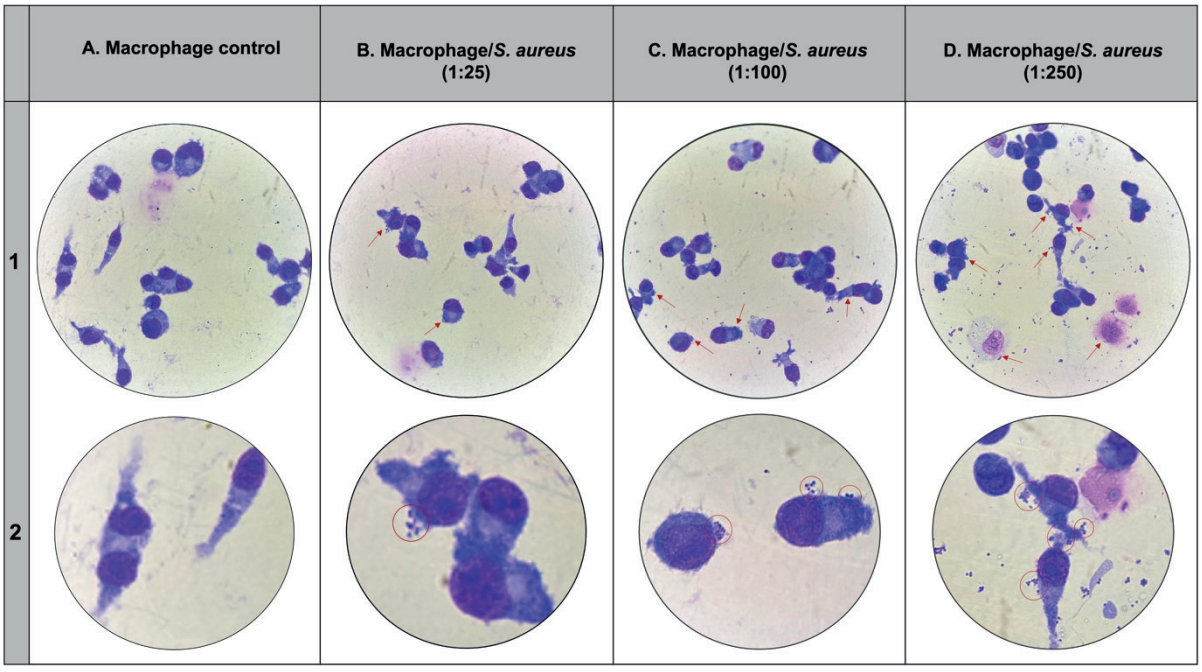

Figure 2. THP-1-derived macrophages infected with S. aureus. A. Uninfected THP-1-derived macrophages, B. Macrophages infected with S. aureus at 1:25 ratio, C. Macrophages infected with S. aureus at 1:100 ratio, D. Macrophages infected with S. aureus at 1:250 ratio.

Figures 1 are shown at 40x magnification and Figures 2 are shown at 100x magnification. Red arrows and circles indicate S. aureus colonies.

RNA isolation was performed to examine the expression levels of 15 circRNA genes in both control and infected groups. It was observed that as the infection rate increased, there was a decrease in the total RNA concentrations. The concentrations and purity values of total RNA isolated from infected and control THP-1-derived macrophages are shown in Table 2.

Table 2. Concentration and Purity Values of total RNA Samples

Experimental Group	RNA Concentration (ng/µl)	A260/280	A260/230
THP-1 Control	141,92	1,799	1,826
THP-1/SA (1:25)	74,2	1,784	1,801
THP-1/SA (1:100)	72,15	1,874	1,794
THP-1/SA (1:250)	21,32	1,785	1,783

The IDs and detailed information of the investigated circRNAs were obtained from the online database http://www.circbank.cn. These circRNAs, the genes from which they are transcribed, their chromosomal locations, genomic lengths, circRNA lengths, and associated functions are listed in Table 3.

Based on RT-qPCR results, 11 circRNAs were found to be expressed in at least one of the experimental groups (Figure 3). However, hsa_circ_0008717, hsa_circ_0043256, hsa_circ_0000285, and hsa_circ_0008603 were not expressed in any group. It was determined that hsa_circ_0025036, hsa_circ_0002483, hsa_circ_0000523, hsa_circ_0008042, hsa_circ_0032822, and hsa_circ_0004771 were expressed

Table 3. Profiles and Functions of Selected circRNAs

CircBase ID	CircBank ID	Chromosomal Location	Genomic Length	circRNA Length	Gene Symbol	Function
hsa_circ_0025036	hsa_circ-FOXM1_010	chr12: 2974520-2975687	1167	174	FOXM1	Induction of cell proliferation and inhibition of apoptosis
hsa_circ_0014130	hsa_circPIP-5K1A_019	chr1: 151206672-151212515	5843	724	PIP5K1A	Inhibition of apoptosis
hsa_circ_0002483	hsa_circPTK2_020	chr8:141874410-141900868	26458	482	PTK2	Induction of cell growth and intracellular signaling
hsa_circ_0008717	hsa_circAB-CB10_009	chr1: 229665945-229678118	12173	724	ABCB10	Induction of cell proliferation, migration and invasion
hsa_circ_0043256	hsa_circACA-CA_033	chr17: 35604934-35609962	5028	483	ACACA	Inhibition of cell proliferation and induction of apoptosis
hsa_circ_0001821	hsa_circT-CONS_00015354_001	chr8: 128902834-128903244	410	410	TCONS 00015354	Cell migration, anti-apoptotic effect
hsa_circ_0000285	hsa_cir-cHIPK3_018	chr11: 33362513-33363232	719	284	HIPK3	Induction of cell proliferation, migration and suppression of apoptosis
hsa_circ_0000523	hsa_circMET-TL3_004	chr14: 21971315-21972024	709	623	METTL3	Inhibition of apoptosis
hsa_circ_0005320	hsa_circSEPT9_001	chr17: 75398140-75398785	645	645	SEPT9	Cell proliferation and cell cycle regulation
hsa_circ_0000869	hsa_circGNG7_001	chr19: 2672918-2673380	462	462	GNG7	Autophagy and cell cycle inhibition
hsa_circ_0008603	hsa_circALD-H3A2_004	chr17: 19554859-19575269	20410	1290	ALD-H3A2	Extracellular vesicles (exosomes) formation mechanism
hsa_circ_0005035	hsa_circIG-F1R_001	chr15: 99250790-99251336	546	546	IGF1R	Inhibition of invasion and migration
hsa_circ_0008042	hsa_circ-CPEB4_009	chr5: 173371969-173380275	8306	680	CPEB4	Induction of cell proliferation
hsa_circ_0032822	hsa_circ-CEP128_021	chr14: 81209418-81244390	34972	595	CEP128	Induction of cell proliferation
hsa_circ_0004771	hsa_circN-RIP1_007	chr21: 16386664-16415895	29231	203	NRIP1	Inhibition of cell proliferation and induction of apoptosis

only in THP-1-derived macrophages. Additionally, the expression level of hsa_circ_0001821, which was also detected in THP-1-derived macrophages, significantly decreased in the 1:25 infection condition ($p<0.01$) and was not detected in other groups.

Interestingly, hsa_circ_000869 expression was remarkably upregulated at the 1:250 infection ratio ($p<0.0001$). On the other hand, hsa_circ_0014130 showed a significant decrease in expression at the 1:250 infection condition ($p<0.0001$). The expression level of hsa_circ_0005320 increased in parallel with increasing infection ratios but was not expressed under the extremely high infection condition of 1:250 (Figure 3).

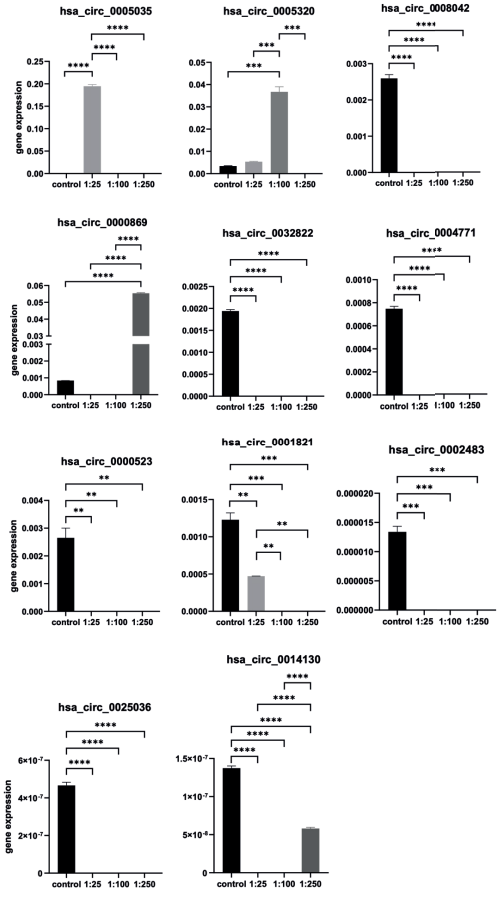

Figure 3. Expression of selected circRNAs in S. aureus-infected THP-1-derived macrophages. (: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$).*

To estimate the potential roles of circRNAs expressed in THP-1-derived macrophages infected with S. aureus, functional analysis was performed. Gene Ontology (GO) analyses indicating the biological processes and associated diseases involving the expressed circRNAs are presented in Figure 4.

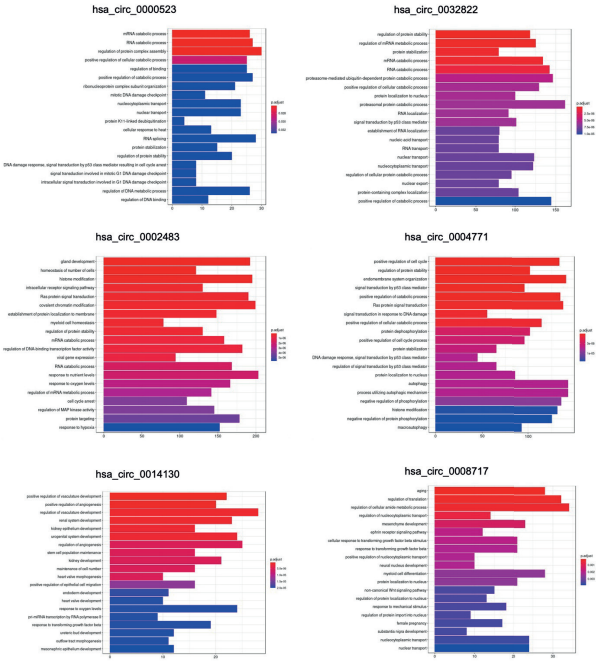

Figure 4. Gene Ontology (GO) analysis of expressed circRNAs. P-values are visualized using color gradients and numerical values.

According to the GO analysis, hsa_circ_0000523, hsa_circ_0032822, and hsa_circ_0002483 were associated with RNA and mRNA catabolic processes, whereas hsa_circ_0002483, hsa_circ_0032822, and hsa_circ_0004771 were involved in the regulation of cellular catabolic processes.

DISCUSSION

Circular RNAs (circRNAs) are a type of single-stranded RNA molecule that form a covalently closed continuous loop. The circular structure of naturally occurring circRNAs confers resistance to exonuclease activity, resulting

in significantly greater stability compared to linear RNAs. Additionally, their ease of detection in body fluids such as saliva, blood, gastric fluid, as well as in tissues and exosomes, makes circRNAs attractive candidates as potential biomarkers for the diagnosis of various diseases[19,20]. Recent studies suggest that dysregulated circRNA expression may contribute to cancer initiation and progression. Moreover, circRNAs are proposed to be promising biomarkers for liquid biopsy-based cancer diagnostics and potential therapeutic targets in cancer treatment[21-23].

Macrophages play a central role in the dynamics of *S. aureus* infections. Understanding the interactions between *S. aureus* and macrophages is therefore crucial for uncovering bacterial survival strategies and host immune defense mechanisms[5,24,25]. However, the expression and functional role of circRNAs in response to *S. aureus* infection remains largely unexplored26. Thus, in this study, we investigated the expression levels and potential functions of selected circRNAs in THP-1-derived macrophages infected with different ratios of S. aureus.

In this study, THP-1-derived macrophages were infected with *S. aureus* at varying MOI ratios to assess circRNA expression dynamics during the acute phase of infection. The data revealed that 11 different circRNAs were expressed in at least one experimental group. Among them, hsa_circ_0025036, hsa_circ_0002483, hsa_circ_0000523, hsa_circ_0008042, hsa_circ_0032822, and hsa_circ_0004771 were expressed exclusively in THP-1-derived macrophages, with hsa_circ_00005035 expressed only in the 1:25 infection group.

A similar study conducted by Xia et al. investigated circRNA expression levels in macrophages after 12, 24, and 48 hours of infection. However, their study used only a single MOI and focused on long-term interactions. They reported minimal circRNA expression overall, with only two circRNAs (hsa_circ_0000311 and chr13:43500472–43544806) showing infection-associated upregulation[17].

In contrast, by using three different infection intensities (low–medium–high) in our study, we aimed to characterize the circRNA response in macrophages as a dose–response pattern, considering that clinical pathogen exposure levels vary. Furthermore, by limiting the infection period to 4 hours, we focused on circRNA expression changes that may be associated with early cellular responses and initial gene regulation. To our knowledge, this is the first study in the literature to combine both MOI diversity and early infection time points in exploring circRNA expression dynamics in macrophage–*S. aureus* interactions. Unlike the findings of Xia et al., we observed significant decreases in the expression of two circRNAs at the early 4-hour time point. Among the MOI conditions, a 1:250 ratio induced the most notable changes in circRNA expression, suggesting that circRNA expression can vary in a dose-dependent manner.

To further investigate the biological roles and potential mechanisms of circRNAs in THP-1-derived macrophages responding to *S. aureus* infection, Gene Ontology (GO) analyses were performed. Based on these analyses, circRNAs involved in RNA/mRNA catabolic processes and regulation of cellular catabolism were not affected by infection, whereas circRNAs known to positively influence cell proliferation were found to be downregulated.

Our results suggest that hsa_circ_0025036, hsa_circ_0002483, hsa_circ_0000523, hsa_circ_0008042, hsa_circ_0032822, and hsa_circ_0004771, whose expression stops with infection, may serve as potential diagnostic biomarkers for *S. aureus*. Further studies are required to elucidate the possible relationships of these circRNAs with the host's initial sensing and signaling responses (e.g., PAMP recognition, inflammasome activation).

The findings of this study indicate that certain circRNAs may be evaluated as potential biomarkers, particularly in sepsis or acute infections involving *S. aureus* exposure. Moreover, the dose-dependent and early-phase expres-

sion changes we observed suggest that these circRNAs could serve as candidates for biomarker discovery, rapid diagnostic development, and infection monitoring in S. aureus-related diseases. Overall, these results represent a significant step forward in understanding host–pathogen interactions and may support the development of innovative therapeutic strategies in infection management.

Ethics Approval

This study was conducted in vitro and did not require ethical approval.

Peer-review

Externally and internally peer-reviewed.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this study.

Funding

This study was supported by the Scientific Research Projects Coordination Unit of Ege University (Project No: TSKBP-2024-31870).

References

1. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339(8):520-532. doi:10.1056/NEJM199808203390806.
2. Tam K, Torres VJ. Staphylococcus aureus Secreted Toxins and Extracellular Enzymes. Microbiol Spectr. 2019;7(2):10.1128/microbiolspec.gpp3-0039-2018. doi:10.1128/microbiolspec.GPP3-0039-2018.
3. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W. Molecular Mechanisms of Drug Resistance in Staphylococcus aureus. Int J Mol Sci. 2022;23(15):8088. doi:10.3390/ijms23158088.
4. Jubrail J, Morris P, Bewley MA, et al. Inability to sustain intraphagolysosomal killing of Staphylococcus aureus predisposes to bacterial persistence in macrophages. Cell Microbiol. 2016;18(1):80-96. doi:10.1111/cmi.12485.
5. Pidwill GR, Gibson JF, Cole J, Renshaw SA, Foster SJ. The Role of Macrophages in Staphylococcus aureus Infection. Front Immunol. 2021;11:620339. doi:10.3389/fimmu.2020.620339.
6. Lacoma A, Cano V, Moranta D, et al. Investigating intracellular persistence of Staphylococcus aureus within a murine alveolar macrophage cell line. Virulence. 2017;8(8):1761-1775. doi:10.1080/21505594.2017.1361089.
7. O'Keeffe KM, Wilk MM, Leech JM, et al. Manipulation of Autophagy in Phagocytes Facilitates Staphylococcus aureus Bloodstream Infection. Infect Immun. 2015;83(9):3445-3457. doi:10.1128/IAI.00358-15.
8. Tuchscherr L, Medina E, Hussain M, et al. Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med. 2011;3(3):129-141. doi:10.1002/emmm.201000115.
9. Thänert R, Goldmann O, Beineke A, Medina E. Host-inherent variability influences the transcriptional response of Staphylococcus aureus during in vivo infection. Nat Commun. 2017;8:14268. Published 2017 Feb 3. doi:10.1038/ncomms14268.
10. Liang Z, Guo W, Fang S, et al. CircRNAs: Emerging Bladder Cancer Biomarkers and Targets. Front Oncol. 2021;10:606485. doi:10.3389/fonc.2020.606485.
11. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression [published correction appears in PLoS Genet. 2013 Dec;9(12). doi:10.1371/annotation/f782282b-eefa-4c8d-985c-b1484e845855]. PLoS Genet. 2013;9(9):e1003777. doi:10.1371/journal.pgen.1003777.
12. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and Characterization of Circular RNAs As a New Class of Putative Biomarkers in Human Blood. PLoS One. 2015;10(10):e0141214. doi:10.1371/journal.pone.0141214.
13. Verduci L, Tarcitano E, Strano S, Yarden Y, Blandino G. CircRNAs: role in human diseases and potential use as biomarkers. Cell Death Dis. 2021;12(5):468. Published 2021 May 11. doi:10.1038/s41419-021-03743-3.
14. Fu Y, Wang J, Qiao J, Yi Z. Signature of circular RNAs in peripheral blood mononuclear cells from patients with active tuberculosis. J Cell Mol Med. 2019;23(3):1917-1925. doi:10.1111/jcmm.14093.
15. Liu H, Lu G, Wang W, et al. A Panel of CircRNAs in the Serum Serves as Biomarkers for Mycobacterium tuberculosis Infection. Front Microbiol. 2020;11:1215. doi:10.3389/fmicb.2020.01215.
16. Phuangbubpha P, Thara S, Sriboonaied P, Saetan P, Tumnoi W, Charoenpanich A. Optimizing THP-1 Macrophage Culture for an Immune-Responsive Human Intestinal Model. Cells. 2023;12(10):1427. doi:10.3390/cells12101427.
17. Xie X, Chen Z, Han M, et al. Profile analysis of circRNAs in human THP-1 derived macrophages infected with intracellular Staphylococcus aureus. Microb Pathog. 2022;165:105466. doi:10.1016/j.micpath.2022.105466.
18. Zhang Y, Shi L, Mei H, et al. Inflamed macrophage microvesicles induce insulin resistance in human adipocytes. Nutr Metab (Lond). 2015;12:21. doi:10.1186/s12986-015-0016-3.
19. Chen Z, Xie Z, Han M, et al. Global Transcriptomic Study of Circular-RNA Expression Profile in Osteoclasts Infected by Intracellular Staphylococcus aureus. Infect Immun. 2023;91(6):e0035722. doi:10.1128/iai.00357-22.
20. Li L, Wang M, Chen Q, et al. Intracellular Staphylococcus aureus infection in human osteoblasts: circRNA expression analysis. Heliyon. 2024;10(7):e28461. doi:10.1016/j.heliyon.2024.e28461.
21. Liu CX, Li X, Nan F, et al. Structure and Degradation of Circular RNAs Regulate PKR Activation in Innate Immunity. Cell. 2019;177(4):865-880.e21. doi:10.1016/j.cell.2019.03.046.
22. Gong Z, Hu W, Zhou C, Guo J, Yang L, Wang B. Recent advances and perspectives on the development of circular RNA cancer vaccines. NPJ Vaccines. 2025;10(1):41. doi:10.1038/s41541-025-01097-x.
23. Zhang N, Wang X, Li Y, et al. Mechanisms and therapeutic implications of gene expression regulation by circRNA-protein interactions in cancer. Commun Biol. 2025;8(1):77. doi:10.1038/s42003-024-07383-z.
24. Long DR, Holmes EA, Lo HY, et al. Clinical and in vitro models identify distinct adaptations enhancing Staphylococcus aureus pathogenesis in human macrophages. PLoS Pathog. 2024;20(7):e1012394. doi:10.1371/journal.ppat.1012394.
25. Bai S, Wen X, Li B, et al. Extracellular vesicles from alveolar macrophages harboring phagocytosed methicillin-resistant Staphylococcus aureus induce necroptosis. Cell Rep. 2024;43(7):114453. doi:10.1016/j.celrep.2024.114453.
26. Yuan H, Liu F, Long J, Duan G, Yang H. A review on circular RNAs and bacterial infections. Int J Biol Macromol. 2023;244:125391. doi:10.1016/j.ijbiomac.2023.125391.