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Expression Profile of circRNA in Lung Cancer Cells Infected with *Staphylococcus aureus*

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INTRODUCTION: Lung cancer is one of the main causes of cancer -related deaths on a global scale and ranks first with approximately 1.8 million deaths according to 2022 data.[1] Tumor formation is shaped not only by genetic and epigenetic changes, but also by microorganism-hycre interactions. The cancer load attributed to infections was reported in 2018 as approximately 2.2 million new cases.[2] This shows that chronic bacterial infections can support inflammatory signaling and tumor progression. *Staphylococcus aureus*, one of these pathogens, is known for adhesion, invasion and immune response modulation to the host cell. It can also affect cell behavior by disrupting the epithelial barrier through toxins.[3] In recent years, it has been suggested that these effects on lung epithelium can re -program processes such as proliferation, apoptosis and migration in cancer cells. On the other hand, Circular RNAs (circRNA) show high stability due to the closed ringing of 5′ and 3′ ends and have functions such as miRNA/protein binding or regulation of gene expression. circRNA played a role in the basic processes of cancer biology such as proliferation, metastasis and drug resistance; It is also reported that it can be used as biomarker and therapeutic target in lung cancer.[4,5] This study aims to examine the circRNA expression changes after infection of A549 (lung adenocarcinoma) and Beas-2b (normal bronchial epithelial) cells with *S. aureus*.

MATERIAL AND METHODS: In order to create an intracellular infection model, A549 and Beas-2B cell lines are infected with *S. aureus* 6538p for 2 hours of 1:50 infection (MOI). It was then treated with gentamicin for 2-24 hours to destroy non-cell bacteria. Intracellular infection was displayed using Giemsa painting method and intracellular *S. aureus* load was measured by CFU analysis, infection index was calculated (infection index = dilution factor × 10). In the 4th and 24th hours of 1:50 infected cells, trizol was insulated with RNA and the concentrations were measured and CDNA was synthesized. circRNA levels were analyzed with reverse transcription quantitative polymerase chain reaction (RT-qPCR). The circular RNA candidates used in the study were determined through CircBank database. The potential target genes and expression levels of these circRNAs were examined in detail using CircAtlas 3.0 database.

RESULTS: In the A549 and Beas-2b cells infected with 1:50 *S. aureus*, the presence of intracellular bacteria was evaluated microscopic with Giemsa dyeing performed at the 4th and 24th hours of infection. According to the infection index analysis at 4 hours of infection, Beas-2B cells were found to be higher than A549 cells. This suggests that normal bronchial epithelial cells may be more sensitive to bacterial internalization. As a result of the RT-qPCR analysis performed in cells after infection, a significant decrease in the expression levels of hsa_circ_0000003,

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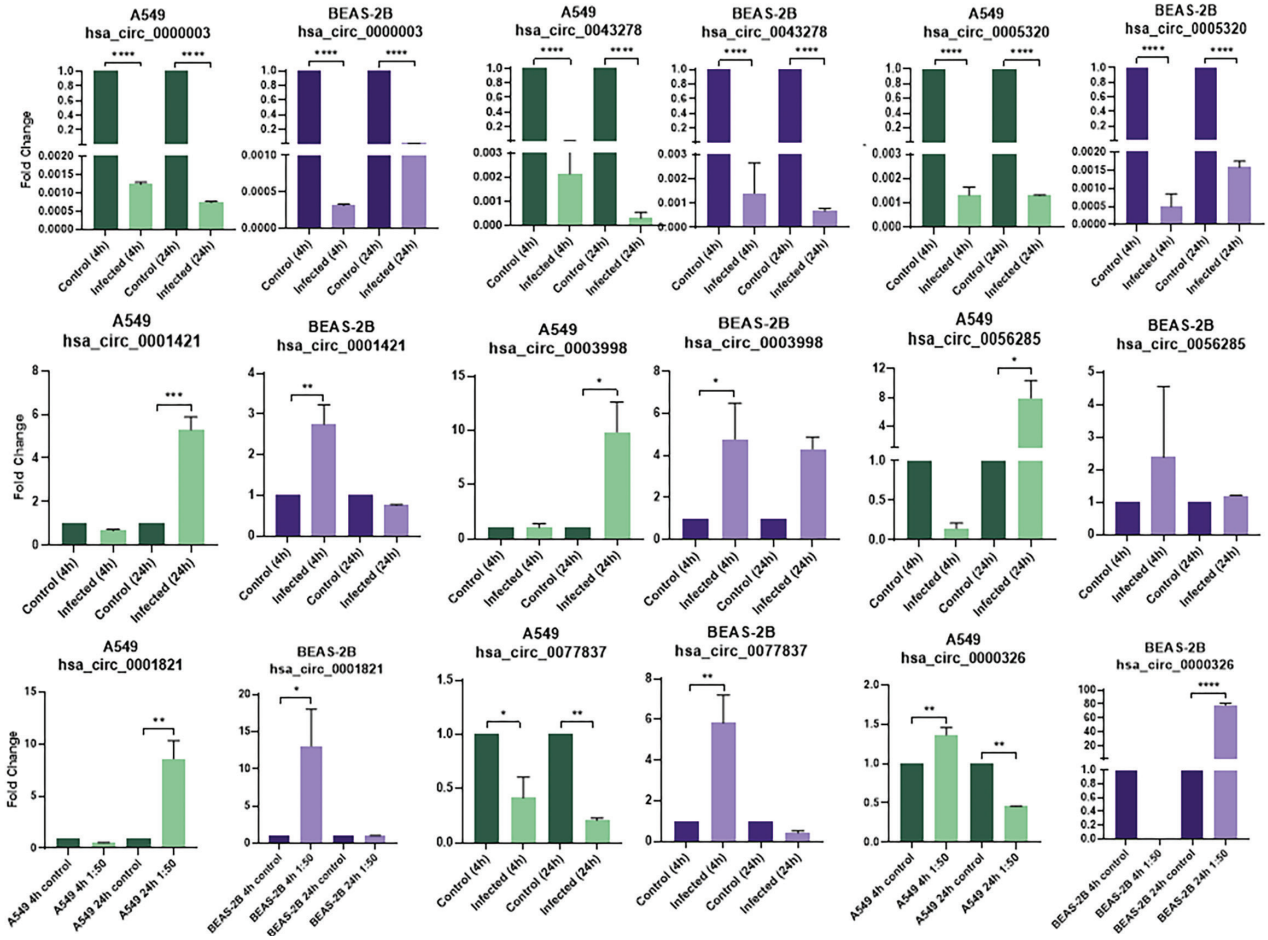

Figure 1. RT-qPCR floor charts after 4 and 24 hours of infection of A549 and Beas-2B cells at a rate of 1:50 ratio
RT-qPCR: Reverse transcription quantitative polymerase chain reaction

hsa_circ_0043278, hsa_circ_0005320, hsa_circ_0001421, hsa_circ_0003998, hsa_circ_0056285, hsa_circ_0001821, hsa_circ_0077837 and hsa_circ_0000326 circular RNAs were observed in both A549 and Beas-2B cells ($P \leq 0.0001$) (Figure 1). These findings show that *S. aureus* infection may suppress the expression of the related circRNA and that these molecules may play a potentially role in the response of infection.

CONCLUSION: In this study, *S. aureus* infection A549 lung cancer and BEAS-2B normal bronchial epithelial cells were successfully achieved and the effects of infection on the circRNA expression profile were evaluated. The data obtained show that infection changes the expression of hsa_circ_0000003, hsa_circ_0043278, hsa_circ_0005320, hsa_circ_0001421, hsa_circ_0003998, hsa_circ_0056285, hsa_circ_0001821, hsa_circ_0077837 and hsa_circ_0000326 CIRCRNA in host cells, and that these molecules can undertake potential regulatory roles in the infection response. These different expressions in the infection process of circRNA can both contribute to a better understanding of disease mechanisms and can be considered as biomarker or therapeutic target in the future.

KEYWORDS: Lung cancer, *Staphylococcus aureus*, circRNA, infection, biomarker

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