P. aeruginosa Quorum Sensing System and the Experimental Model of Chronic Respiratory Infection: Scientific Letter

P. aeruginosa Quorum Sensing Sistemi ve Deneysel Kronik Akciğer Enfeksiyonu Modeli

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ABSTRACT Opportunistic pathogen *Pseudomonas aeruginosa* is a model microorganism used in explaining cell-to-cell signaling system, named Quorum Sensing (QS). In chronic *P. aeruginosa* infections, bacteria survive in biofilm and defend themselves against host defense mechanisms by different virulence factors. Three known QS systems, *las, rhl* and quinolones are basically responsible for the production of virulence factors. Experimental animal models are commonly used in examining in vitro and in vivo behaviors of the bacteria, and explaining interactions between host, bacteria and antibacterials. Among these, chronic pulmonary infection model, applied by administration of agar-impreagnated bacterial suspensions into trachea, is special because it mimicks the biofilm, a virulence factor. Comparative studies conducted particulary with QS wild-type and QS mutant strains, provide important information for understanding the pathogenesis of infection. In this article, the role of *P. aeruginosa* QS system in the experimental model of chronic lung infection is focused.

Key Words: *Pseudomonas aeruginosa*; quorum sensing; biofilms; models, animal

ÖZET Fırsatçı bir patojen olan *Pseudomonas aeruginosa,* Quorum Sensing (QS) olarak adlandırılan hücreden hücreye iletişim sistemlerini açıklamada model mikroorganizma olarak kullanılmaktadır. Kronik *P. aeruginosa* enfeksiyonlarında bakteri, biyofilm içerisinde yaşamını sürdürmekte ve çeşitli virülans faktörlerinin yardımı ile konak savunma sisteminden korunmaktadır. Bu virülans faktörlerinin üretiminden ise temel olarak *las, rhl* ve kinolon olmak üzere üç QS sistemi sorumlu tutulmaktadır. Bakterinin in vitro ve in vivo davranışlarının incelenmesi, bakteri, konak ve antibakteriyeller arasındaki etkileşimin açıklanmasında hayvan modelleri sıklıkla kullanılmaktadır. Bu modeller arasında bakterinin agar içine emdirilmesi ve trakeaya inokulasyonu ile gerçekleştirilen kronik akciğer enfeksiyonu modeli, virülans faktörlerinden biyofilmi taklit etmesi nedeni ile ayrı bir yere sahiptir. Özellikle QS vahşi tip ve QS mutant suşlar ile yapılan karşılaştırmalı çalışmalar, enfeksiyonun patogenezinin anlaşılmasında önemli bilgiler sunmaktadır. Bu yazıda *P. aeruginosa*'nın QS sistemine ve bu sistem üzerinden bakterinin deneysel kronik akciğer infeksiyonu modelindeki etkilerine odaklanılacaktır.

Anahtar Kelimeler: *Pseudomonas aeruginosa*; çoğunluk algısı; biyofilmler; modeller, hayvan

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Determining local bacterial density through communication signals, and accordingly coordinate behaviors is interpreted as Quorum Sensing (QS). QS systems that are regulated by cell-density-dependent signal molecules, coordinate plasmid conjugation, biofilm formation and production of various pathogenity factors.[1] In chronic infections, a number of pathogenic bacteria, including *Pseudomonas aeruginosa*, live in

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biofilm (microcolonies surrounded by exopolysaccharides) to escape from the host cell response and survive under the changing environmental conditions, when attach to biotic and/or abiotic surfaces. It is noted that bacteria growing in biofilm may slow down their metabolism depending on the the oxygen amount at the biofilm base, and develop resistance to antibiotics more compared to planctonic bacteria. Biofilm formation increases mortality and morbidity rates, and economical costs of treatment; therefore it is among the major research subjects that should be widely concentrated on.[2,3] Bacteria living as a community are noted to be more pathogenic to the host. *P. aeruginosa* has been used as the model microorganism in the investigations conducted on biofilm formation, which is a population-level virulence factor, and on QS systems.[4]

P. aeruginosa, with various virulence factors and emerging antibiotic resistance, is a common infectious agent causing high mortality and morbidity rates.[5,6] In chronic *P. aeruginosa* infections, bacteria survive in biofilm to prevent themselves from host response. Controlling various extracellular virulence factors secreted by *P. aeruginosa* and the biofilm production are shown to be regulated by three interrelated QS systems. They are defined as; *las*, *rhl* and quinolone systems.[7]

The primary system is regulates Las B elastase production, thus named as *las* system; and consists of *las I* gene (3-oxo-C12-HSL-L, AI synthase gene responsible of long chain AHL synthesis) and *las R* gene (encoding "transcriptional activator" protein). This system regulates biofilm formation and production of extracellular virulence factors such as Las B elastase, Las A protease, and exotoxin A.[8] The secondary QS system, *rhl,* comprises *rhl I* gene (C4-HSL, AI synthase gene, short chain AHL) and *rhl R* gene (encoding "transcriptional activator" protein). This system regulates production of Rhl AB operon (operon: regulatory DNA region), synthesis of "rhamnosyltranspherase" enzyme, which is required in rhamnolipid production, and synthesis of Las B elastase, Las A protease, pyocyanine, cyanide, and alkaline protease.[9] The other system, AHQ signals include 2-heptyl-3-hydroxy-4-quinolone (Pseudomonas Quinolone Signal, PQS) and 2-heptyl-4-quinolone (HHQ).[10-12] PQS is synthesized via the pqsABCDE operon, which is responsible for generating multiple Aqs, including 2-heptyl-4-quinolone (HHQ), the immediate PQS precursor. In addition, PQS signaling plays an important role in *P. aeruginosa* pathogenesis because it regulates the production of diverse virulence factors including elastase, pyocyanin and LecA lectin, also influences biofilm formation.[10,13]

Quorum sensing in *P. aeruginosa* consists of a complex network. Although *las* system has been shown to have a particular role among these cell-density-dependent signal molecules, hierarchial mechanisms have also been demonstrated in the context of both stimulating *rhl* system and regulating PQS production.[14] If the *las* system fails to function, QS systems would be re-organized, such that the secondary system, *rhl* may substitute the *las* system.[10] The PQS system is intricately connected to the AHL systems. The *rhl* and *las* systems exert negative and positive regulation mechanisms on PQS, respectively, while PQS has positive influences on the *rhl* system.[15] Moreover, it has been recently reported that, in the case of *las* activation problem, residual transcriptions of rhlI and rhl may interact with environmental factors and activate *rhl* regulator, which may consequently lead to delayed activation of *las* regulator.[16]

EXPERIMENTAL ANIMAL MODELS IN UNDERSTANDING QS SYSTEM

Although there are studies conducted with cell culture method and examine the importance of the QS system and various virulence factors that are controlled by the QS system concerning pathogenesis of *P. aeruginosa* infections,[17] experimental animal models also have significantly important roles in examining in vivo behaviors of bacteria, and in explaining the interaction between bacteria, host and antibacterials.

Experimental model of chronic pulmonary infection is among the widely preferred models. Nasal or intratracheal administration of bacterial suspension to the animal is the generally used method to develop pulmonary infection.[18,19] However,

bacteria are directly exposed to the host defense system in this method, so they can be eradicated from the body within a short period of time, therefore it may not always be an effective method in the process of generating pulmonary injury.

Agar beads model, which is utilized by Cash et al.[20] through administrating agar-impregnated bacterial suspensions into the trachea, is a higly accepted experimental model used in generating chronic pulmonary infection with *P. aeruginosa*. In the experimental modeling, the purpose of impregnating bacteria into the agar is, by mimicking the presence of biofilm, to prevent direct exposure of bacteria to the host defense system, which would allow bacteria to survive in the airways, hence generate chronic pulmonary infection. With this model, histopathological changes such as goblet cell hyperplasia, focal necrosis, acute and chronic inflammatory cell infiltration and cytokine accumulation, that may be seen in chronic pulmonary infection caused by *P.aeruginosa* are specified as being mimicked.[20,21] These histopathological changes can also be used in determining lung injury score, therefore can provide statistical data with regard to the pulmonary effects of the investigated virulence factors in the comparison of the experimental groups. Criteria developed by Jerng et al.[22] are commonly used in scoring lung injury severity. Accordingly, histopathological preparations of lung tissue stained with hematoxylin-eosin (H&E) staining are examined under the light microscope, and capillary congestion, hemorrhage, neutrophil infiltration and thickening of the alveolar septum are evaluated, and the degree of injury is scored between 0-4, where 0, no damage, 1; mild damage 2; moderate damage, 3; severe damage and 4, maximal damage. Although agar beads method is ideal in examining the pulmonary effects of biofilm in the development of pulmonary infections, it is required to be performed by experienced researchers because of the implantation difficulties of agar beads and high mortality rates due to mechanical obstruction during application.

In understanding the pathogenesis of *P. aeruginosa* infections, comparative studies conducted with the QS wild-type strains and the mutated strains of QS system, in which signal molecule-producing genes (*lasI* and *rhlI*) are found to be mutated, provided significant information. In the chronic pulmonary infection model developed by Imamura et al.[23] by using QS wild type (PAO1) and three QS mutant strains (*ΔlasI, ΔlasI/ΔrhlI, ΔrhlI*), it is stated that the QS mutant strains can be eradicated from lungs more effectively compared to wild-type strains, therefore they cause less damage to the lungs. Wu et al.[24] developed *P. aeruginosa* pnemonia in rats by using PAO1 and mutant PAO JP2 (*ΔlasI/ΔrhlI*) strains. In the early stages of infection, researchers observed considerably faster and stronger immune response against mutant strain, larger amount of pulmonary IFN-g, and more powerful response of polymorphonucleer leukocytes as well as more rapid antibody response, and they concluded that functional *lasI* and *rhlI* genes have significant roles in the severity of pulmonary infections. In the study carried out with clinical specimens, Karatuna and Yagci noted that even though QS had a key role in the respiratory tract infections caused by *P. aeruginosa*, the QS mutant strains having low sensitivity to antimicrobial agents might also lead to infection.[25] Karaman et al. developed pulmonary infection model in rats by using PAO1 ve PAO JP2 (*ΔlasI/ΔrhlI*) reference laboratory strains with agar beads method.[26] As a result of the quantitative cultivation of bronchoalveolar lavage (BAL) and lung tissues on the 14th day of the infection, researchers reported a significant increase in the bacterial count of the group infected with wild-type strains compared to the group infected with mutant strains. In this situation, it can be interpreted as *lasI* and *rhlI* mutant strains may be less virulent, therefore may be eradicated effectively by the host defense system.

All these data distinctively suggest the importance of QS system in *P. aeruginosa* infection, however the capability of QS mutant strains to develop infections indicate the need to conduct further investigations on this subject. Experimental animal models are supposed to guide these research studies.

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