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Novel understandings of host cell mechanisms involved in chronic lung infection: *Pseudomonas aeruginosa* in the cystic fibrotic lung

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ABSTRACT

As one of the most debilitating and underdiagnosed hereditary conditions across the globe, cystic fibrosis requires intensive support from the healthcare system – particularly due to the increased susceptibility to chronic infection and resulting respiratory failure which can rapidly lead to death. In turn, the prevalence and action of a certain strain of bacterium – *Pseudomonas aeruginosa* – has gained a great deal of interest. Life-threatening chronic infections by *P. aeruginosa* have been shown to involve biofilm formation, proliferation and the release of quorum-sensing signaling molecules. Understanding the mechanism cascade by which this strain attacks cells within the respiratory epithelium, most notably airway epithelial cells, could offer insight into the pathway and components, which are attractive targets for therapeutic interventions.

Introduction

Cystic fibrosis (CF) is a severely underdiagnosed genetic disorder in several areas of the world, especially Asia, which disrupts the respiratory, digestive and reproductive systems via the production of abnormally thick mucus linings or biofilms leading to fatal lung infections [1]. In CF, a mutant strain of *Pseudomonas aeruginosa* form biofilms that sustain chronic infections. Quorum sensing (QS) mechanisms are intimately involved in intercellular communication in bacterial networks that regulate density detection and biofilm formation. The objectives of this review paper are to (i) evaluate the unprecedented role of QS signaling molecules in signaling pathways of airway epithelial cells that enhance *P. aeruginosa* biofilm survival and chronic infection and (ii) identify strategies for therapeutic intervention based on current understandings of these pathways.

P. aeruginosa in CF and lung infections

In CF pathophysiology, the dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel in airway epithelial cells promotes a lung environment, which is largely susceptible to chronic infection and inflammation – the leading cause of death in CF patients [2]. More specifically, a genetic mutation in the CF gene produces a deficient CFTR protein, which is incapable of secreting chloride ions, ultimately altering the ion balance in the lung. This leads to increased water reabsorption and increased mucus production resulting in dehydration and reduced mucocilliary clearance of the lung, which creates the ideal environment for infection by bacterial colonies [3]. Interestingly, sputum isolates from the lungs of CF patients revealed only a few bacterial agents responsible for chronic infection [3]. *P. aeruginosa*, an antibiotic-resistant strain, was the most abundant and recurrently observed in all ages of CF patients. In contrast, other bacterial strains such as *Staphylococcus aureus* and *Haemophilus influenzae*, were also seen, but predominantly in patients in their first or second decade of life and less abundantly in later stages of life. Additionally, *P. aeruginosa* utilizes a number of mechanisms to attenuate the innate immune response and augment the inflammatory response, facilitating biofilm survival and thus chronic infection.

Biofilm formation and QS mechanisms

Introducing *P. aeruginosa* into a pathological CF lung nearly always results in its mutation to a biofilm-forming and sustaining mucoid strain [4]. Biofilms are clusters of matrix-encased microcolonies that colonizes, layers, and adheres to a surface [5]. In the context of CF, *P. aeruginosa* biofilms can form and adhere to the total exposed surface of epithelial cells forming the lung wall. The formation of biofilms and regulatory QS pathways has extensively been characterized within *P. aeruginosa* gene networks. QS involves

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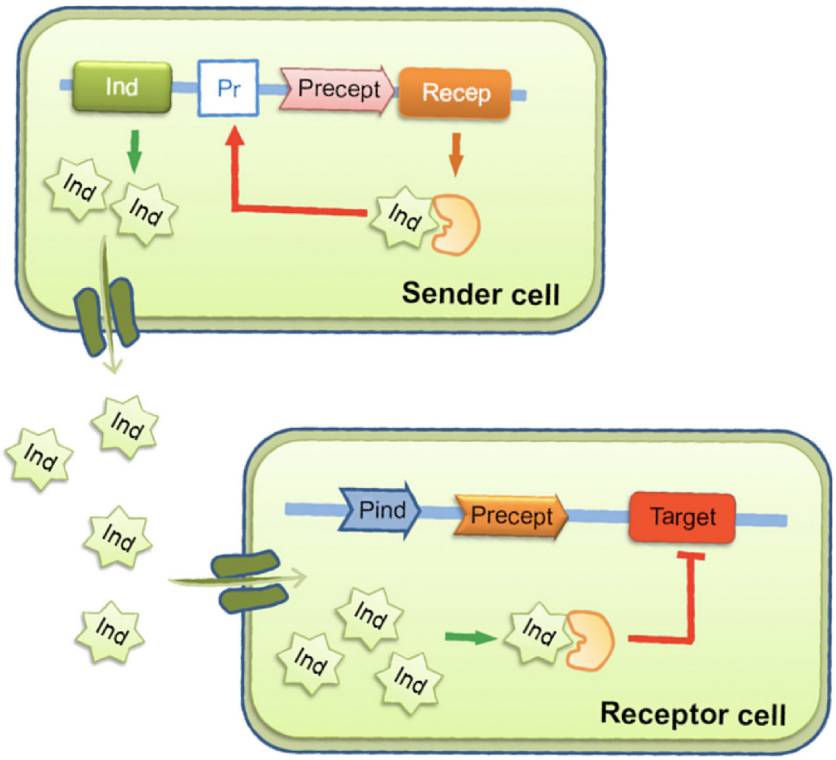

Fig. 1. Design of quorum sensing in a colony of bacteria. The sender cell synthesizes a QS molecule (AHLs or AQs in *P. aeruginosa*), which acts as an inducer that stimulates its receptor in the receptor cell. Increased binding of inducer to an adapter in the receptor cell can cause the inducer-adapter complex to inhibit target region which allows for density detection and arrests biofilm formation. Ind: inducer; Pr: promotor; Precept: receptor's promoter. Adapted from *Trosset & Carbonell*, 2015 and made available under the terms of the Creative Commons Attribution-NonCommercial 3.0 Unported License, under Dove Medical Press Limited.

the synthesis of an inducer or signaling molecule, which diffuses from a sender cell to a receiver cell, which allows for cell-to-cell communication essential for density detection and biofilm growth (Fig. 1). Once the inducer binds the activating protein in the receiving cell, it can interact with the chromosomal genetic sequence to promote the production of an inducer synthase to signal density to neighboring cells. In *P. aeruginosa*, there are three QS intercellular signaling pathways: *las*, *rhl*, and *pqs* (Fig. 2). In *las* and *rhl* signaling, the inducers are *N*-acylhomoserine lactones (AHLs) while QS mechanisms for *pqs* employ 2-alkyl-4-quinilones (AQs) as inducers [6]. Although independent systems, there is a complex interplay and ample crosstalk between genes and gene products as depicted in Fig. 2. Despite the detailed characterization of virulence of *P. aeruginosa*, the actions of QS inducer molecules on host cell signaling pathways regulating immune responses, especially in CF, remain largely unexplored and are thus still poorly understood.

Cytotoxicity of QS signals in nonpolarized cells

The functional importance of polarity in airway epithelial cells is demonstrated by cytotoxic effects of the AHL, *N*-3-oxo-dodecanoyl-L-homoserine lactone (C12), in altering integrity of the epithelium and decreasing gap junctional intercellular communication between epithelial cells [7]. When C12 isolated from *P. aeruginosa* was administered to cultures of bronchial epithelial cells in polarized and nonpolarized conditions *in vitro*, polarized cells were unaffected while nonpolarized cells had both reduced cell integrity and lower gap junction channel conductivity rendering the cells increasingly prone to physical damage and incapacitate its ability to coordinate signalling and response. Furthermore, these effects of exogenous C12 were suppressed by the application of Src tyrosine family inhibitors and Rho-associated protein kinase (ROCK) in nonpolarized cells, suggesting these signalling molecules may be involved in the cellular cascades that C12 acts through (Fig. 3). It has been proposed this pathway activates transcription and translation of genes involved in cytoskeletal remodelling [7]. In addition to these differences, the study also revealed that degradation of C12 was also impaired in the nonpolarized cells. Notably, there was an increase in expression levels of intracellular paraoxonase (PON2), a catalyst for this degradation process, in polarized cells when compared to unpolarized cells. This further indicates that the loss of polarity of cells could result in the accumulation of C12. This is particularly significant in infected CF patients where the dysfunction of chloride conductance can impair the polarity of airway epithelial cells by promoting the imbalance of ions between the apical and basolateral surfaces and thereby result in increased vulnerability of cells and diminished response to infection [8]. With the novel identification of these enzymatic molecules involved in cell signalling, it is now conceivable that utilizing Src tyrosine and ROCK inhibitors or targeting the activation of PON2 degradation of C12 may alleviate detrimental effects on the epithelium in CF patients [7].

QS inducers decrease NRF2-bound antioxidant response element

In an even more recent experiment, QS molecules from the biofilm presented a decrease in NRF2 transcription factor expression [9]. NRF2 is typically involved as a transcription factor in responding to inflammation and oxidative stress where reactive oxygen species (ROS) accumulate (Fig. 4). In the normal NRF2 pathway, NRF2 remains bound to Keap1, which promotes polyubiquination and as a result, signals degradation of the complex by proteasomes in the cell [9,10]. When exposed to oxidative stress, the sulfide bridges formed by cysteine residues involved in anchoring NRF2 to Keap1 become oxidized leading to the release

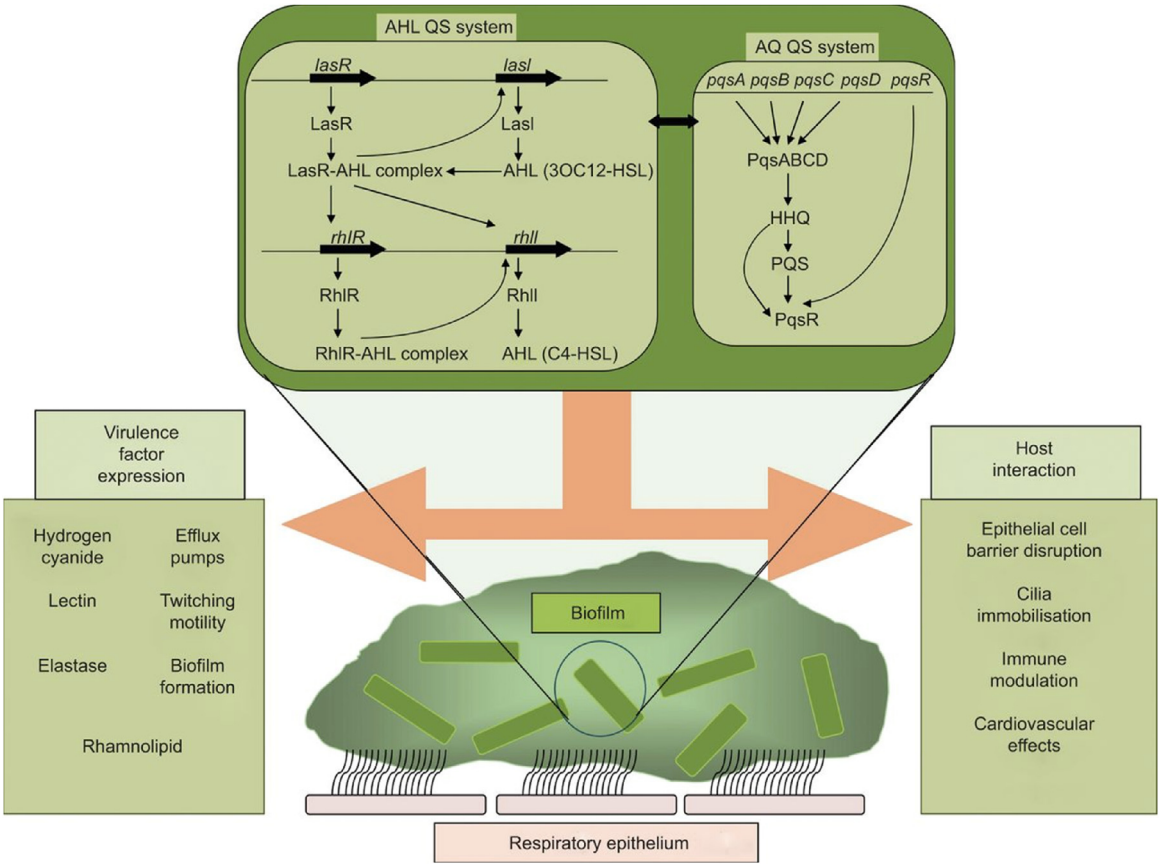

Fig. 2. Quorum sensing (QS) pathways of *Pseudomonas aeruginosa*. *P. aeruginosa* uses *N*-acylhomoserine lactones (AHLs) and alkylquinolines (AQ) mediated QS systems to control the production of virulence factors and the interaction with the host. The balance between these signalling mechanisms is also a key determinant in biofilm formation. 3OC12-HSL: *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (C12); C4-HSL: *N*-butanoyl-L-homoserine lactone; HHQ (AQ): 2-heptyl-4-quinolone; PQS: *Pseudomonas* quinolone signal. Reproduced with permission of the ©ERS 2018: *European Respiratory Journal Sep 2016, 48 (3) 664-673; DOI: 10.1183/13993003.00436-2016*.

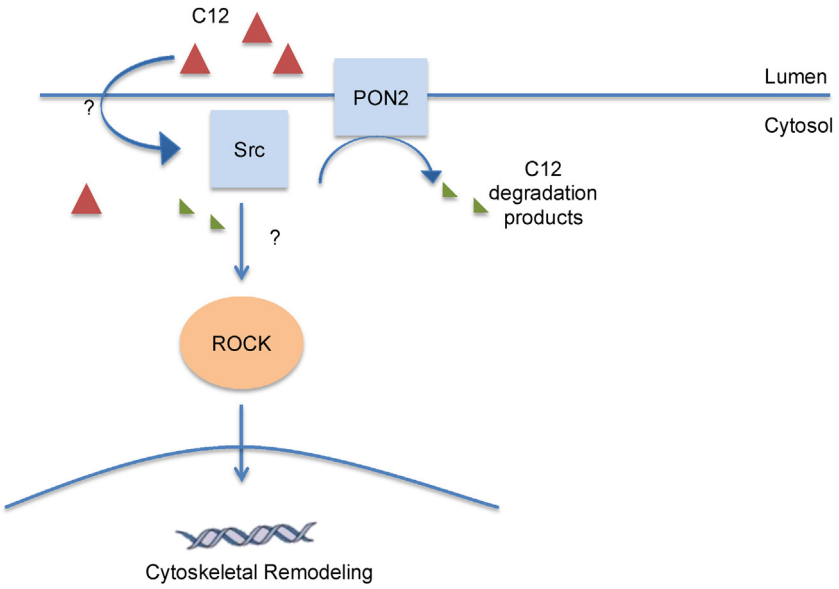

Fig. 3. Proposed pathway for cytoskeletal remodeling altering structural integrity in epithelia and decreasing conductance in gap junctions. The QS signaling molecule C12 is involved either indirectly or directly in the activation of a Src tyrosine family kinase, which can then act through various pathways to activate ROCK. The ROCK enzyme can then be used for genetic modifications within the nucleus that can interfere with structural integrity by cytoskeletal remodeling. Alterations in genetic transcription and translation have also been proposed as modifier of gap junction conductance in the cell. In this pathway, PON2 is a membrane protein that acts intracellularly to catalyze degradation of C12 thereby preventing actions of C12 on the pathway.

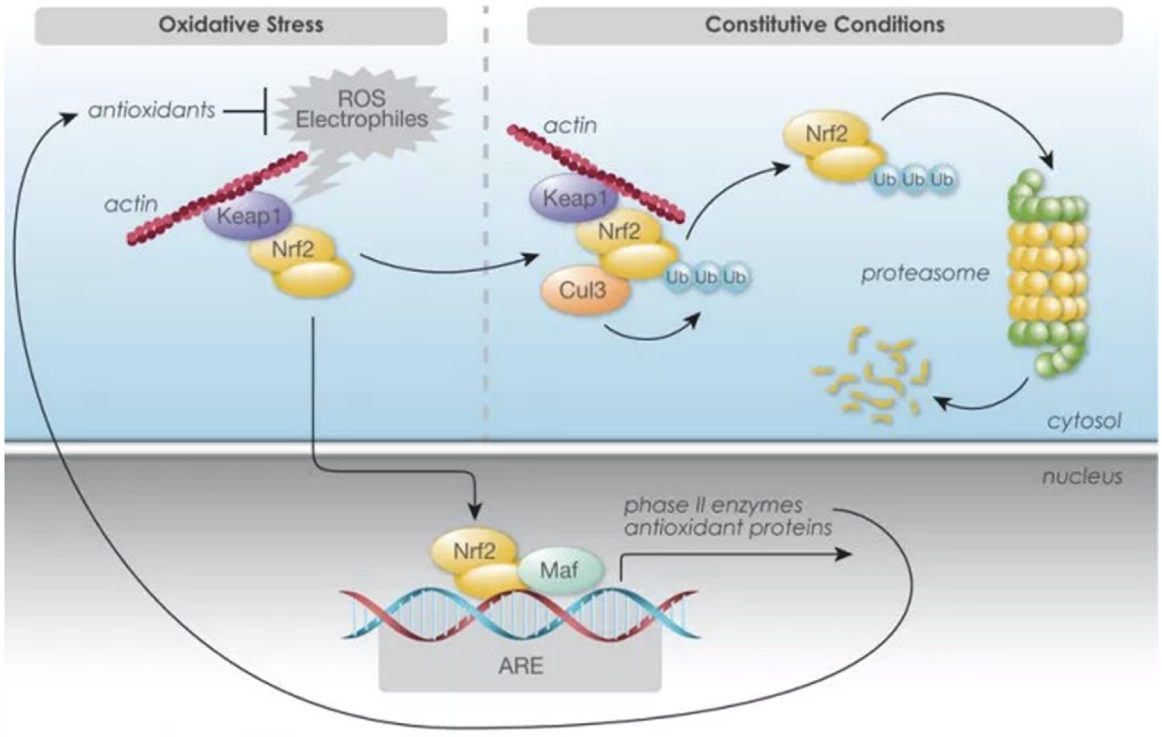

Fig. 4. The Keap1-Nrf2 antioxidant pathway. Typically, when confronted with oxidative stressors, cells must quickly augment their antioxidant capacity to counteract increased ROS production and maintain homeostasis. The nuclear factor erythroid 2-related factor (Nrf2) is a transcription factor that functions as the key controller of the redox homeostatic gene regulatory network. Under oxidative and electrophilic stresses, the Nrf2 signaling pathway is activated to enhance the expression of a multitude of antioxidant and phase II enzymes that restore redox homeostasis. Kelch-like ECH-associated protein 1 (Keap1), a cysteine-rich protein that is anchored to actin in the cytosol, interacts with Nrf2, acting as an adaptor protein for the Cul3-dependent E3 (Cul3) ubiquitin ligase complex. Under normal conditions, Keap1 promotes ubiquitination and eventual degradation of Nrf2. Rapid turnover maintains a low, basal level of Nrf2. The many cysteine residues in the amino acid sequence of Keap1 enable it to act as a sensor, detecting changes in cellular redox state. An increase in intracellular ROS or electrophiles yields an increase in the oxidation or conjugation of key Keap1 cysteines, which weakens its activity as an E3 ligase adaptor. Thus, during cellular stress, Keap1 is less effective at promoting Nrf2 degradation. As Nrf2 is stabilized it enters the nucleus where it activates transcription of a host of cytoprotective genes, including the components of an antioxidant system that can balance high ROS levels. Reproduced with permissions from Cayman Chemical: *May, O. (2012).* Nrf2 Antioxidant Stress Response [online] *Caymanchem.com. Available at:* https://www.caymanchem.com/news/nrf2-antioxidant-stress-response *[Accessed 16 Oct. 2018].*

and stabilization of NRF2. Consequentially, NRF2 can enter the nucleus to promote transcription and translation of antioxidant enzymes and phase II proteins such as hemeoxygenase-1 (HO-1) and NADH quinone dehydrogenase which convert reactive products to a more stable conjugate form and increase expression of interleukin-8 (IL-8) in the cell, which is crucial to immune response [9]. The effect of *P. aeruginosa* infection in this pathway was studied using clinical isolates of biofilm from a CF patient (CFTRdelF508 homozygous), which were co-cultured with immortalized human bronchial epithelial BEAS-2B cell line. An untreated PAO1 strain and a PAO1 ΔlasI mutant strain, which lacks the genetic machinery to synthesize C12 synthase were also used. In this mutant strain, the *lasI* mutation does not only inhibit C12 production. In fact, it will also interferes with other QS signaling mechanisms as LasI can regulate *pqs* synthesis of AQs and C12 binding to LasR will stimulate *rhl* pathways to synthesize other AHLs [6]. Therefore, a *lasI* knockout would not differentiate between the effects of C12 or any other QS signaling molecules. Following co-culture of these *P. aeruginosa* strains with BEAS-2B cells, a significant decrease in NRF2 transcriptional activity was observed in CF patient co-culture but no differences between untreated and mutants strains. This suggests that QS signaling molecules are indirectly or directly responsible for the decrease in NRF2 activity. In light of cellular mechanisms in CF governing NRF2 and Keap1 signaling pathways during infection by *P. aeruginosa*, the AHLs and AQs have been suggested to act in a similar mechanism to ROS in the oxidative stress conditions. Ultimately, this would trigger the cascade of events that lead to enzyme synthesis and IL-8 production that can respond to the oxidative and cytotoxic effects of QS signaling molecules [8,11]. Based on this cellular characterization, a number of effective therapeutic strategies can be considered. First, the potentiation of NRF2 activity is one promising intervention, which could counteract the biofilm activity and respond to detrimental QS signals affecting the host. Alternatively, Nrf2-Keap1 dissociation can be activated or polyubiquination and degradation of the complex can be blocked. Another possible form of treatment may be to enhance the activity of HO-1 and NADH quinone dehydrogenase to enhance the clearance of reactive molecules and enhance the immune response.

Implications and future directions

Characterization of complex signaling pathways involved in *P. aeruginosa* QS-mediated mechanisms is integral to the development of novel therapeutics to address pathologies such as CF. The recent findings elucidating the cytotoxicity of QS signals in the non-polarized lung epithelium and NRF2 interference have advanced current understandings of cellular mechanisms underlying pathophysiology that sustain chronic infection and mediate its adverse effects. As a result, this has opened doors to more strategic interventions, which can guide us closer to more effective treatments and perhaps a cure for CF.

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Competing interests

None declared.

Ethical approval

Not required.

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