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Persister Cells: When Bacteria Go to Sleep: A Minireview

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ABSTRACT

Persister cells are phenotypic variants within bacterial populations, exhibiting remarkable tolerance to various antibiotics. These cells enter a dormant state, allowing them to withstand high antibiotic concentrations. Upon antibiotic removal, these lingering persister cells transition back to normal cells through phenotypic switching. Toxin and antitoxin systems play a pivotal role in persister formation, inducing hibernation through mechanisms such as interference with replication, translation, and reduced proton motive force. The development of persister cells is influenced by growth phases and environmental stresses. Biofilms harbor a higher abundance of persister cells compared to planktonic cultures, with the biofilm matrix offering protection. Persister cells are implicated in recurring and chronic infections, posing a significant public health threat. Consequently, there is an urgent need to comprehensively understand persister cell formation and physiology, leading to the development of drugs and strategies for effective eradication.

المثابرات: عندما تنام البكتيريا

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الكلمات المفتاحية
المضاد الحيوي
الغشاء الحيوي
العدوى المزمنة
علاج المثابرات

الملخص

المثابرات هي متغيرات ظاهرية للخلايا الطبيعية في مجموعات البكتيريا وهي متسامحة للغاية مع العديد من المضادات الحيوية. لا تزال المثابرات في حالة خاملة وقادرة على النجاة من جرعات عالية من العلاج بالمضادات الحيوية. لكن بعد إزالة المضادات الحيوية، تبدأ في التطور كخلايا طبيعية نتيجة التبديل الظاهري. تلعب أنظمة السموم/مضادات السموم دورًا مهمًا في تكوين هذا النمط من الخلايا. يؤدي تنشيط السموم إلى سبات الخلايا من خلال مجموعة متنوعة من الآليات، بما في ذلك التداخل مع النسخ المتماثل والترجمة وانخفاض القوة الدافعة للبروتون، ويتأثر تكوين المثابرات بمراحل النمو والضغوط البيئية. الخلايا المثابرة أكثر وفرة في الأغشية الحيوية منها في المزارع البكتيرية أو ما يسمى بالعوالق، حيث تحمي مواد الغشاء الحيوي المثابرات من الخلايا المناعية للمضيف، يُعتقد أن الخلايا المثابرة مسؤولة عن الالتهابات المتكررة والمزمنة، والتي تشكل تهديدًا كبيرًا على الصحة العامة. لذلك، هناك حاجة ملحة لفهم تكوين الخلايا المثابرة وفسيولوجيتها بشكل كامل وتطوير الأدوية والاستراتيجيات للقضاء على الخلايا المثابرة

Introduction

Persisters are dormant variants of normal cells that form stochastically in bacteria and other microbial populations, exhibiting a high tolerance to antimicrobials [1]. The concept of persisters dates back to 1944 when Joseph Bigger, a doctor at the University of Dublin, conducted pioneering research on the mechanism of penicillin's action. Bigger observed that penicillin lysed a growing population of *Staphylococcusspp*. [2]. "Intriguingly, a smaller number of persistent cells survived this lysis, prompting Bigger to spread the resulting clear fluid onto the surface surrounding the area and document the formation of surviving colonies." [3]. Upon reinoculation, these colonies developed into a culture that exhibited re-lysis in the presence of penicillin but gave rise to a new, small subpopulation, which Bigger termed "persistent" to distinguish them from resistant mutants [1].

Harris Moyed dedicated his efforts to addressing this problem well into the 1980s, conducting a targeted search for persistent genes in selected mutants exhibiting higher levels of persistence. This was achieved through repetitive exposure of *E. coli* growing with ampicillin. Subsequently, the surviving cells were allowed to develop in the absence of antibiotics. Following multiple cycles of this process, two types of colonies were identified after plating on solid medium. The first comprised conventional antibiotic-resistant mutants capable of growing in the presence of ampicillin. The second group consisted of mutants that produced persistent cells at an increased rate but were unable to grow in the presence of ampicillin [3,4].

The intermittent application of high doses of bactericidal antibiotics to a population of chemically mutagenized bacteria facilitated the isolation of stable hip mutants (high persistence) [5,6]. One such persistent mutation has been linked to the hipA gene. Notably, the allelic strain hipA7 produced approximately 1% of persisters that survived ampicillin treatment in exponential cultures, representing a persistence level approximately 1,000 times higher than that of the wild-type strain [4].

In 2000, Kim Lewis rediscovered persistent cells while investigating the time-dependent destruction of bacteria in

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biofilms [7].

The difference between antibiotic tolerant Persisters cells and the resistant cells

The resistant bacterial cells employ various resistance mechanisms to survive, including target modification through mutation, target modification via specialized enzymatic changes, target substitution (such as expressing an alternate target), antibiotic modification or destruction, and limited permeability to antibiotics [8,9]. All these mechanisms share the common goal of preventing the antibiotic from binding to its target. Each resistance mechanism enables cells to grow at high concentrations in the presence of the antibiotic [4].

In contrast to resistant cells that can grow in the presence of antibiotics, persister cells do not exhibit growth under antibiotic exposure. These persister cells constitute a small fraction of exponentially growing cells but are present in significant numbers during the stationary phase and in biofilms [4]. Importantly, persistent cells may play a crucial role in chronic infections. The conformation of persister cells is not well understood, and the metabolic state of these cells remains uncertain [8–10].

When and how does it form (Persisters cells formation mechanism)

The excessive and careless use of antibiotics has resulted in the emergence of antibiotic resistance in a broad spectrum of pathogenic bacteria [11]. Antibiotic resistance mechanisms stem from genetic changes that hinder antibiotic activity, leading to resistance. In other words, resistant cells can grow in the presence of antibiotics [12,13], whereas Persister cells remain dormant and do not undergo growth. Persister cells are believed to be less susceptible to antibiotics because they avoid cellular activities that would make them more vulnerable to the drugs [10].

Persistent cells constitute a subpopulation of total cells and are composed of various pathogenic bacterial species. These cells display tolerance to antibiotics and other environmental stressors [14]. Persistent cells arise following a temporary transition of cells from an antibiotic-sensitive state to a slow or non-growing (dormant) state characterized by reduced metabolism and physiological activity [15].

Nonetheless, these cells return to a growing state once the antibiotic treatment is discontinued [16]. Persistent dormant cells found in biofilms or planktonic cell populations have demonstrated tolerance. The biofilm offers additional resistance by preventing the penetration of antibiotics [13,17].

Persistent cells are formed through various mechanisms, including stochastic events in growing cell cultures, environmental factors (such as nutrient restriction, changes in carbon sources during diauxic growth, oxidative stress, DNA damage, and exposure to subinhibitory antibiotic concentrations), host-pathogen interactions, and intra-species communication through the Quorum Sense Ratio (QS) system [5,18,19]. Biofilm represents another environmental condition that fosters the development of persistent cells [13].

Lastly, a recent study has elucidated another mechanism of persistent formation involving "low energy" levels, i.e., decreased levels of cellular ATP concentration [20,21]. According to this mechanism, the emergence of persistence is linked to the fact that most antibiotics target processes related to energy production within bacterial cells [5].

The hipA gene is the first gene found to impact the formation of persistent cells [3,22]. The primary driver for the formation of persistent cells is this toxin-antitoxin (TA) pair, commonly found on plasmids and bacterial chromosomes [23] (although their role is largely unknown). These TA pairs, specifically the toxin component, play a crucial role as they induce a state of dormancy, enabling cells to evade the effects of antibiotics [13]. Prokaryotic TA loci encode two components: a stable "toxin" (always a protein) that inhibits cell growth and disrupts an essential cellular process (e.g., translation via mRNA degradation) and a labile "antitoxin" (RNA or protein) that regulates the activity of the toxin [5,24,25].

The genetic architecture and the nature of the regulation of toxin-antitoxin (TA) activity resulted in the classification of TA into five classes. Type I and III TA loci code for small antitoxin RNAs. In contrast, type IV antitoxins protect toxin targets instead of directly inhibiting the toxin. Type V antitoxins are site-specific endoribonucleases that inhibit the expression of the toxin by cleaving mRNAs that encode the toxins [5,26,27].

The biofilm and its relation with Persisters cells

Biofilm is a complex structure of the microbiome, consisting of a single type of cell in a group or several bacterial colonies that adhere to a surface. These cells are embedded in extracellular polymeric substances [29], a matrix typically composed of eDNA (extracellular DNA), proteins, and polysaccharides, exhibiting high resistance to antibiotics [30]. Infections associated with biofilms include common ailments like middle ear infections and gingivitis in children. The most well-known biofilm-producing organisms are those causing infections in internal devices (*Staphylococcus aureus* ,*E. coli*, *Pseudomonas aeruginosa* [31].

Approximately 80% of chronic and recurrent bacterial infections in the human body are attributed to bacterial biofilms. Microbial cells within biofilms have demonstrated 10-1,000 times greater resistance to antibiotics compared to planktonic cells, which are bacterial communities that adhere and proliferate on surfaces and are covered with an exopolymeric matrix [32].

Nonetheless, planktonic cells originating from these biofilms are, in most cases, entirely sensitive to antibiotics. It's crucial to note that biofilms don't actively grow in the presence of high antibiotic concentrations, so they don't exhibit facilitated resistance compared to planktonic cells [33]. Most cells within a biofilm remain highly susceptible to bactericidal agents, such as fluoroquinolone antibiotics or metal oxyanions, capable of eliminating both rapidly dividing and slow-moving or non-growing cells [34].

However, the exopolymeric matrix of the biofilm provides protection against immune cells [35]. Additionally, the lingering bacteria within the biofilm can withstand both antibiotic therapy and immune system attacks. As antibiotic concentrations decrease, persisters can repopulate the biofilm, thereby outlasting new planktonic cells [33]. The challenge of biofilm resistance to most therapies likely stems from the issue of persistence [4].

Treatment strategies against persistent cell formation

Various strategies have recently been developed involving the use of natural and chemically synthesized anti-persistent compounds that directly kill or reactivate persistent cells [36,37]. There are four treatment strategies: The direct killing of metabolically dormant Persister cells. In this strategy, the cellular envelope and cellular DNA are the primary targets. The integrity and potential of the bacterial cell membrane or wall are particularly crucial barriers to the target [38]. Persister cells' tolerance against the lethal effects of

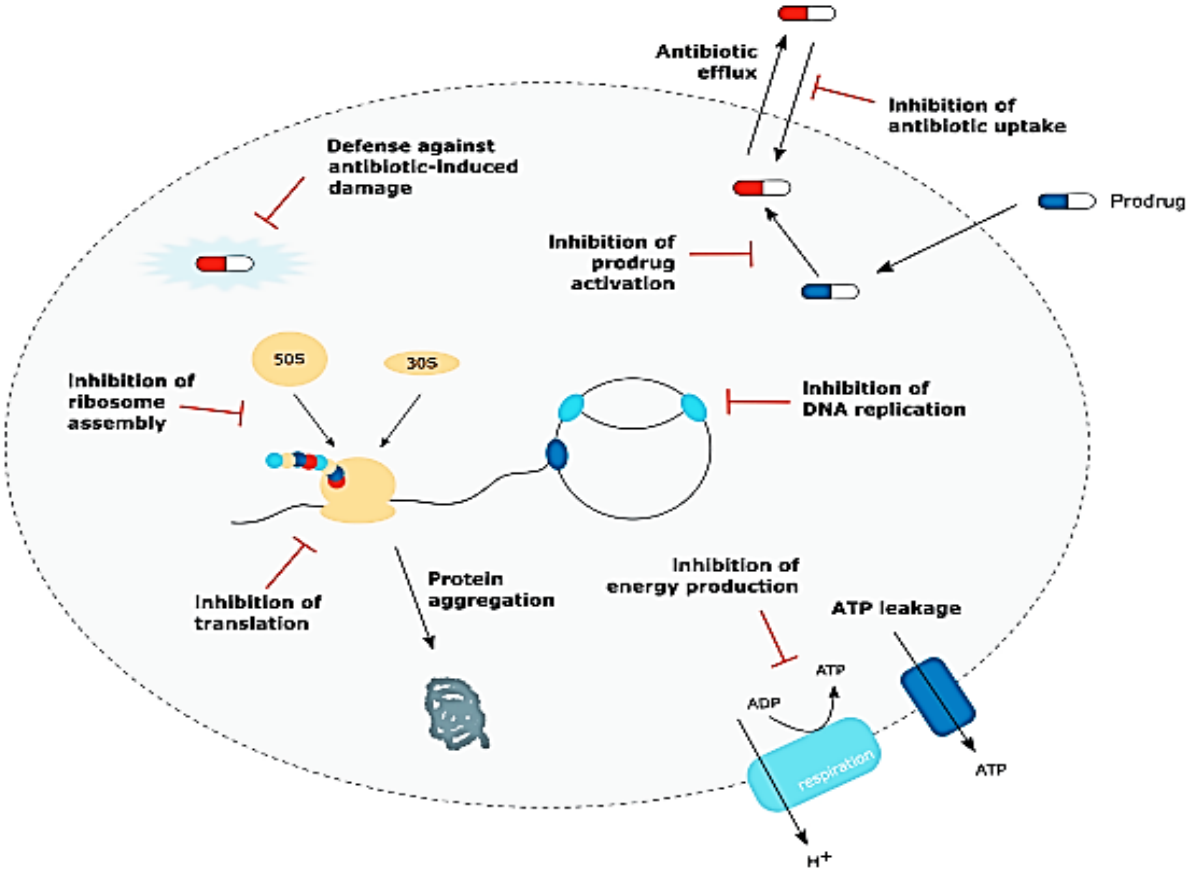

Fig. 1: Persisters cells formation mechanisms [28].

antimicrobial drugs is attributed to their ability to temporarily terminate or delay multiple cellular activities [39]. Small molecules and phages, combined with recombinant proteins, can form an electrostatic interaction with the oppositely charged cell membrane and wall components, thereby changing the membrane potential and damaging the physical integrity of the cell [7]. The direct killing strategy can also be executed using physical methods such as blue light in the wavelength range of 400-470 nm, shown to inactivate a broad spectrum of bacterial cells through the generation of reactive cytotoxic oxygen species after photoexcitation of intracellular photosensitizers such as porphyrins and flavins [40]. Bacteriophages are viruses that infect and replicate within bacterial cells; they can be exploited as bacteriolytic agents in cases where antimicrobial agents fail to kill Persister cells [41].

Awakening or sensitization of Persister cells into metabolically active and antibiotic-susceptible states: Most antibiotics are effective against metabolically active cells because they target components of nucleic acid metabolism, protein synthesis, and cytoplasmic membranes [42]. Treating persistent cells with antibiotics has been challenging, mainly due to their inactive metabolism. Certain types of sugar molecules, as well as glycolytic pathway intermediates such as pyruvate, may trigger the transition of Persister cells to an antibiotic-susceptible state when used as sole carbon sources [7].

Combination therapies: This involves combining the anti-persistent drug with conventional antibiotics and non-antibiotic drugs to revitalize the effectiveness of antibiotics and diversify the effects of the anti-persistent [43].

The complex nature of biofilms and persistent cells presents significant challenges to conventional single-objective antibiotic treatment strategies [44]. Components of the cell wall or membrane can lead to decreased concentrations of antibiotics in the biofilm [45,46]. Even when the antibiotic can penetrate the biofilm matrix, tracking its circulation and controlling the release at the infection site is a challenging task that requires frequent antibiotic application and the use of specialized tracking systems [47]. Consequently, there has been a recent surge in interest in combination drug therapies as an alternative treatment strategy. Options include antibiotics and/or antimicrobial compounds, as well as non-drug delivery agents [7].The complex nature of biofilms and persistent cells presents significant challenges to conventional single-objective antibiotic treatment strategies [44]. Components of the cell wall or membrane can lead to decreased concentrations of antibiotics in the biofilm [45], [46]. Even when the antibiotic can penetrate the biofilm matrix, tracking its circulation and controlling the release at the infection site is a challenging task that requires frequent antibiotic application and the use of specialized tracking systems [47]. Consequently, there has been a recent surge in interest in combination drug therapies as an alternative treatment strategy. Options include antibiotics and/or antimicrobial compounds, as well as non-drug delivery agents [7].

Other indirect approaches involve interfering with the Quorum Sensing (QS) signaling circuit and genetically engineering the metabolic pathways of persister cells [7]. Pathogenic biofilm-forming bacteria communicate using chemicals called QS signaling molecules [48]. The QS signaling circuit is a promising target for controlling persistent cell formation during biofilm formation. Strategies

include degrading QS signaling molecules and inhibiting pathways that produce QS signaling molecules [49,50]. According to a recent study, the phenol-soluble modulin toxin (PSM) increased the sensitivity of persistent *S. aureus* cells by damaging the bacterial membrane through its lytic activity. The production of the PSM toxin by S. aureus is directly regulated by the Agr QS system [51,52].Other indirect approaches involve interfering with the QS signaling circuit and genetically engineering the metabolic pathways of persister cells [7]. Pathogenic biofilm-forming bacteria communicate using chemicals called QS signaling molecules [48]. The QS signaling circuit is a promising target for controlling persistent cell formation during biofilm formation. Strategies include degrading QS signaling molecules and inhibiting pathways that produce QS signaling molecules [49,50]. According to a recent study, the PSM increased the sensitivity of persistent *S. aureus* cells by damaging the bacterial membrane through its lytic activity. The production of the PSM toxin by *S. aureus* is directly regulated by the Agr QS system [51,52].

Several industrial applications of genetic engineering have led to significant improvements in the production of valuable metabolites from microorganisms. However, cells that persist in a metabolically inactive state have been neglected in terms of engineering the metabolic pathways that control their persistence [53,54]. While few research studies have focused on engineering the metabolic pathway to control persistent cell formation, folate products play a crucial role in DNA synthesis and the production of methionine and NADPH [7,55]. A recent study by Morgan and others demonstrated that disrupting folate biosynthetic pathways reduces the ability of bacteria to develop persistent cells [56]. Additionally, antifolate drugs as antimicrobials offer another approach to controlling persistent cell formation. The disruption of carbamoyl phosphatesynthetase, a metabolic enzyme involved in the synthesis of the amino acid arginine and the pyrimidine nucleotide base, leads to a reduction in persistent cell formation [57,58].

Conclusions and perspectives

The presence of persister cells in transiently dormant and slow metabolism states has been linked to multidrug tolerance of bacteria within biofilms as well as the recalcitrant nature of infections. Treatments against Persisters cells observed in biofilms or planktonic cell populations generally fail due to the complex nature of Persisters cells and biofilms, which are beneficial to each other on a multitude of levels. As the clinical importance of Persister cells with respect to treatment failures is increasingly acknowledged, novel therapeutic strategies against these cells and their associated infections are urgently needed. Since Persister cells play a significant role in biofilm relapse and non-healing wound infections, the development of "persistercides" will become just as significant as the growing research area of anti-biofilm agents against chronic infections. To replace the outdated conventional antibiotic monotherapies, a significant number of alternative treatment strategies against Persister cells have been developed that employ physical agents and numerous naturally occurring or chemically synthesized compounds. The mechanisms employed by antimicrobial peptides such as bacteriocins, either alone, in combination with other agents, or as adjuncts to antibiotics, to awaken Persister cells or inhibit their formation are another promising area of research**.**

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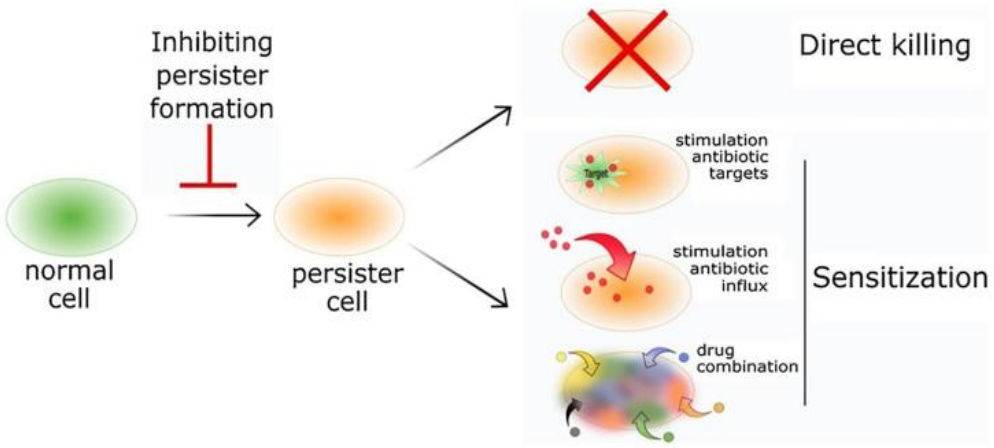

Fig. 2: Schematic illustration of the strategies used to treat persister cells; one mechanism targets inhibitors before persister formation, and the other two either directly kill inhibitors or make them susceptible to antibiotic [58].

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