CRISPR-Cas9 System Mediated Targeting of Toxin-Antitoxin Systems for Combating Multidrug Tolerance in Bacterial Pathogens

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Abstract: Unlike resistance, tolerance arises from physiological changes rather than genetic mutations in a subpopulation of microorganisms. Bacterial toxin-antitoxin (TA) systems are having well established role in multidrug tolerance. This multidrug tolerance arises due to small subpopulation of microbial cells, called persister cells. Persister cells can survive even at lethal concentration of multiple antibiotics and leads to chronic and recurrent infections. Certain infectious diseases including tuberculosis, endocarditis and cystic-fibrosis caused by *Mycobacterium tuberculosis, Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively are typically chronic in nature. Moreover, implanted medical devices such as catheters and artificial joints and indwelling devices such as orthopedic prostheses and heart valves are also sites of chronic infections where reoperation is the only method for treating the infection. In addition, urinary tract infections and unhealing wounds are also example of chronic infections. The therapeutics that is currently available suppresses, but do not eradicate this infection completely. Furthermore, there are two major challenges of presently available broad-spectrum antibiotics, indiscriminate eradication of bacteria and the selective pressure for resistance. These challenges can be addressed by CRISPR-Cas9 engineering & targeting. CRISPR loci preserve a molecular record of earlier encountered bacteriophages & plasmids. It also maintains short CRISPR-derived RNAs (crRNAs) guide Cas9 proteins that are complementary to targets for sequence-specific degradation. In this study, we targeted parE goxin of parDE toxin-antitoxin system in *Pseudomonas aeruginosa* by CRISPR-Cas9 system. The protein coded by parE toxin is a potent inhibitor of cell proliferation. We constructed the expression vector to enable CRISPR-Cas9 based genome engineering: The expression vector expresses both CRISPR-Cas9 enzyme & sgRNA molecules. For demonstration of functionality of CRISPR-Cas9 system in *P. aeruginosa parE* toxin gene was deleted from *P. aeruginosa* chromosomal region. Owing the modularity & simplicity CRISPR-9 engineering, toxin-antitoxin genes that are play a major role in multidrug tolerance can be targeted. In this way, we can understand the molecular mechanism of persister cell formation that leads to multidrug tolerance in *P. aeruginosa* & other bacteria. Moreover, this system can be utilized to target other bacterial species at the same time & numerous sequences of the same bacterium to prevent the rise of resistant mutant. Along with toxin-antitoxin systems, plethora of antibiotic resistance & virulent determinants can also be targeted & offering the possibility to shape complex bacterial populations.

Keywords: Crispr-Cas9, toxin-antitoxin system, multidrug tolerance, persisters, *Pseudomonas aeruginosa*.

Recent Publications:

1. **Ravi Kumar Chaudhary**, Girraj Singh, Ram Naraian and Siya Ram. Structural and functional in-silico analysis of toxin-antitoxin proteins in persister cells of *Pseudomonas aeruginosa*. Plant *Archives* Vol. 18 No. 2, **2018** pp. 1643-1651
2. **Ravi Kumar Chaudhary**, Girraj Singh, Siya Ram. Homology Modeling and *in silico* Structural Annotation of the Toxin-Antitoxin Systems in *Pseudomonas aeruginosa. Indian Res. J. Genet. & Biotech. 10(1): 180-188 Feburary (**2018**)*
3. Aman Chandra Kaushik, Ajay Kumar, Vivek Dhar Dwivedi, Shiv Bharadwaj, Sanjay Kumar, Kritika Bharti, Pavan Kumar, **Ravi Kumar Chaudhary**, Sarad Kumar Mishra. Deciphering the Biochemical Pathway and Pharmacokinetic Study of Amyloid βeta-42 with Superparamagnetic Iron Oxide Nanoparticles (SPIONs) Using Systems Biology Approach. *Molecular Neurobiology*, **2017**, 1-13 **(*corresponding author**)
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5. Parasuraman Padmanabhan, Ajay Kumar, Sundramurthy Kumar, **Ravi Kumar Chaudhary**, Balázs Gulyás. Nanomaterials in practice for molecular imaging applications: an overview. *Acta Biomaterialia*, **2016**, 4: 1–16.



I am working in the field of molecular biology and nanotheranostics. The main emphasis of my work is to study bacterial persister cells, the role of the toxin-antitoxin system in their formation and development of nano-encapsulated drug based anti-persister therapy. Bacterial populations produce a small number of dormant persister cells (~1%) that exhibit multidrug tolerance. Multidrug tolerance or antibiotic tolerance is the ability of a disease-causing microorganism to resist killing by antibiotics or other antimicrobials. The genesis of my study is in the root of the need for potent & effective anti-persister therapy for eradication of bacterial persisters to provide better treatment options for chronic infections in the near future. I am also involved in the development of nanotheranostics approaches for early diagnosis, imaging & targeting neurodegenerative diseases. The main emphasis of my work is to develop multifunctional nanoparticles and reporter gene imaging.

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