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ORAL ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Enhancement of Human Neutrophil Bactericidal Activity through ROS Regulation

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Antimicrobial agents are vital for treating infectious diseases, but their widespread use has led to a concerning rise in drug-resistant bacteria. The development and overuse of new antimicrobial agents have paradoxically given rise to even more drug-resistant strains. If current trends persist, drug-resistant infections may become the leading global cause of mortality by 2050. However, current measures such as restricting antimicrobial use and promoting appropriate usage have not provided a definitive solution. Therefore, exploring alternative treatment approaches is essential.

In this study, our research was based on a novel strategy that shifts the focus from targeting specific bacterial species to leveraging the defense mechanisms of human neutrophils. Neutrophils play a critical role in innate immunity by engulfing bacteria and generating reactive oxygen species (ROS) in phagosomes. The primary ROS producer in neutrophils is the NADPH oxidase (NOX) complex centered on gp91 ^{phox} (NOX2), a membrane protein of the NOX family. However, excessive ROS are harmful, necessitating negative regulation. Neutrophils contain a negative regulator of reactive oxygen species (NRROS), which regulates ROS production by competing with p22 ^{phox} for binding to NOX2 when inflammation go into ending. NRROS knockout mice have shown increased ROS production and bactericidal activity during bacterial infection, resulting in significantly prolonged survival compared to wild-type mice. If this mechanism could be applied to human cells to enhance bactericidal activity, it would eliminate the need to target specific bacterial species and could revolutionize the treatment of infectious diseases.

We used human induced pluripotent stem cells (iPSC) as a model to validate the NRROS knockout effect in human cells, overcoming challenges in isolating peripheral blood neutrophils due to their limited lifespan, poor in vitro culture tolerance, and genetic editing complexities. We successfully generated NRROS-heterozygous knockout (hetero-KO) and NRROS-homozygous knockout (homo-KO) iPSC clones using genome editing techniques. Additionally, using the PiggyBac system, we established doxycycline-inducible NRROS expression clones (homo-KO+Dox) based on homo-KO clones. These iPSC were differentiated into neutrophils and evaluated for function.

The isolated neutrophil fraction exhibited characteristic segmented nuclei in all clones. We measured ROS production and examined changes induced by NRROS knockout after stimulation. Immediate superoxide production showed no difference between wild-type and NRROS-KO clones. However, after 2 hours of stimulation, the NRROS-KO clones exhibited significantly elevated hydrogen peroxide production, indicating increased total ROS production, possibly due to delayed NOX complex degradation. Furthermore, we assessed the bactericidal capacity of iPSC-derived neutrophils against multidrug-resistant bacteria, revealing significantly enhanced bactericidal activity in NRROS-KO clones compared to wild-type clones.

In conclusion, NRROS knockout in human neutrophils enhances bactericidal capacity through heightened ROS production, offering promising prospects for innovative infectious disease treatments. If successfully harnessed, this innovative treatment modality could revolutionize infectious disease management, combating drug-resistant infections, and improving global health outcomes.

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