Exosomes Deliver Sepsis Treatment in Mice

According to a new study, a novel sepsis treatment that uses exosomes loaded with super-repressor IκB to prevent expression of a transcription factor that drives uncontrolled inflammation helped mice with sepsis live longer and recover from organ damage. The treatment significantly lowered proinflammatory markers but not an anti-inflammatory cytokine, suggesting it could be applied during the early phase of sepsis, before the immune system is suppressed. These findings may also prove valuable in the search for therapies to treat patients suffering from serious COVID-19 infections. “Sepsis or septic shock is highly associated with COVID-19 mortality,” says Kyungsun Choi, an author on the study. “Managing this and other clinical complications is the key to improving disease prognosis in severely ill COVID-19 patients. We strongly believe that our super-repressor-loaded exosome can be an effective and safe therapeutic candidate for severely ill COVID-19 patients.” Sepsis remains the leading cause of mortality in intensive care units, manifesting as systemic inflammation caused by an overactive immune response to infection. Although this syndrome kills 400,000 to 600,000 patients each year in the US and Europe, there are currently no available treatments designed specifically to treat sepsis. To explore a potential treatment option, Choi et al. used a previously developed technology to load proteins expressed from a human embryonic kidney cell line onto exosomes, delivering the treatment in two mouse models of sepsis. The exosomes successfully transported their cargo to neutrophils and macrophages involved in the sepsis inflammatory response, showing therapeutic effects even when the treatment was introduced 1 h after inducing sepsis in the mice. (Sci Adv. 6, eaaz6980; https://doi.org/10.1126/sciadv.aaz6980.)

Unconstrained Genome Targeting with CRISPR-Cas9 Variants Is Less Reliant on PAM

Addressing a fundamental limitation in CRISPR-Cas genome editing, researchers have developed new engineered Cas9 variants that nearly eliminate the need for a protospacer adjacent motif known as PAM. This motif is otherwise required for DNA-targeting CRISPR enzymes. According to the report, the new Cas9 enzymes open up virtually the entire genome for targeting. This expands the potential of CRISPR-Cas systems, the authors say, something they showed by using their approach to correct mutations associated with human diseases located in previously “un-editable” regions of the genome. DNA-targeting CRISPR-associated enzymes find their targets by recognizing PAM sequences—short bits of genetic code that flag editable sections of DNA and serve as a binding signal for specific CRISPR-Cas nucleases. Without an adjacent, recognizable PAM sequence, a Cas enzyme will neither recognize nor successfully attach to and cleave a desired section of DNA. While different Cas enzymes, including variants of the canonical Streptococcus pyogenes Cas9 (SpCas9), recognize different PAM sequences, much of the genome remains un-editable for editing or more prone to generating off-target mutations. Thus, the PAM requirement represents a significant limiting barrier for applications that require high-resolution genome targeting. To address this limitation, Walton et al. engineered new variants of the SpCas9 enzyme capable of targeting and editing sequences bearing a wider array of PAMs. They report on two significant variants: SpG, which is capable of targeting an expanded set of NGN PAMs, and a near-PAM-less variant called SpRY. Collectively, SpG and SpRY enable unconstrained targeting using CRISPR-Cas9 nucleases across nearly the entire genome and with single base pair precision. Using SpRY, the authors were able to correct mutations associated with human diseases located in previously “un-editable” regions of the genome. (Science. Published online 26 March 2020; https://doi.org/10.1126/science.aba8853.)

Study Shows CRISPR Effectiveness against Colitis Pathogen

Clostridioides difficile is an important nosocomial pathogen responsible for 500,000 cases of C. difficile infection (CDI) and 29,000 deaths each year in the US. A recent study shows that the CRISPR-Cas system can be used to effectively target and eliminate these specific bacteria, which cause colitis—a chronic, degenerative disease of the colon. In a proof-of-concept study published in the journal mBio, researchers were able to show pathogen reductions in experiments conducted both in vitro and in mice. The researchers used a bacteriophage to carry a programmable CRISPR to specifically target and eliminate C. difficile bacteria. Use, and overuse, of antibiotics increases susceptibility to C. difficile infection, as antibiotics wipe out both good and bad bacteria in the gut. Relapses occur in some 30% of human patients treated with a standard antibiotic to eliminate C. difficile. In the lab, the CRISPR-Cas systems effectively killed C. difficile in vitro. After that, the researchers tested the approach in mice infected with C. difficile. Two days after the CRISPR treatment, the mice showed reduced C. difficile levels, but those levels grew back 2 days later. Next steps include retooling the phage to prevent C. difficile from returning after the initial effective killing. The researchers said that future work will also involve developing a library of different phages for various C. difficile strains. (mBio 11, e00019-20; https://doi.org/10.1128/mBio.00019-20.)
Gene Therapy Reverses Heart Failure in a Mouse Model of Barth Syndrome

Barth syndrome is a rare metabolic disease in boys caused by the mutation of a gene called tafazzin or TAZ. It can cause heart failure and also weakens the skeletal muscles, undercuts the immune response, and impairs overall growth. There is no cure or specific treatment, but new research suggests that gene therapy could prevent or reverse cardiac dysfunction. The findings, involving new mouse models of Barth syndrome, were published recently in the journal *Circulation Research*. The workers used two models of Barth syndrome. In one, the TAZ gene was deleted in cells throughout the body, and, in the other, the TAZ gene was deleted just in the heart. Most mice with the whole-body TAZ deletion died before birth, apparently because of skeletal muscle weakness. But some survived, and these mice developed progressive cardiomyopathy, in which the heart muscle enlarges and loses pumping capacity. Their hearts also showed scarring, and, similar to human patients with dilated cardiomyopathy, the heart’s left ventricle was dilated and thin-walled. Mice lacking TAZ just in their cardiac tissue, which all survived to birth, showed the same features. The researchers then used gene therapy to replace TAZ, injecting an engineered virus under the skin (in newborn mice) or intravenously (in older mice). Treated mice with whole-body TAZ deletions were able to survive to adulthood. TAZ gene therapy also prevented cardiac dysfunction and scarring when given to newborn mice and reversed established cardiac dysfunction in older mice—whether the mice had whole-body or heart-only TAZ deletions. Further tests showed that TAZ gene therapy provided durable treatment of the animals’ cardiomyocytes and skeletal muscle cells, but only when at least 70% of heart muscle cells had taken up the gene. (Circ Res. 126, 1024–1039; https://doi.org/10.1161/CIRCRESAHA.119.315956.)

Experiments in Mice and Human Cells Shed Light on Nanoparticle Therapy for Cancer

Researchers in the cancer nanomedicine community debate whether use of nanoparticles can best deliver drug therapy to tumors passively or actively through use of a targeting agent to bind to specific cancer cell receptors and, in theory, keep the nanoparticle in the tumor longer. Now, new research on human and mouse tumors suggests the question is even more complicated. Laboratory studies testing both methods in six models of breast cancer found that nanoparticles coated with trastuzumab, a drug that targets human epidermal growth factor receptor 2 (HER2)-positive breast cancer cells, were better retained in the tumors than unmodified nanoparticles, even in tumors that did not express the HER2 protein. A description of the work was published recently in *Science Advances*. It has been known for some time that nanoparticles, when injected into the bloodstream, are picked up by macrophages and other immune system cells. Researchers have been focused on trying to reduce interactions with immune cells because they have been trying to increase the circulation time of the nanoparticles and their retention in tumor cells. However, the new study demonstrates that the immune cells in the tumor collect and react to the particles in such a way to stimulate an anti-cancer response. The investigators applied both unmodified iron oxide nanoparticles and others coated with trastuzumab to five human breast cancer cell lines in vitro, finding that the amount of binding between the trastuzumab-coated nanoparticles and cells depended on how much the cancer cells expressed HER2. The researchers reported that responses were surprisingly different in animal models. In separate experiments, the team used the nanoparticles in two immune-deficient strains of mice engrafted with cells from five human breast cancer cell lines—two that were HER2 negative and three that were HER2 positive. When they studied the animals’ tumors 24 h later, they noticed that nanoparticles coated with trastuzumab were found in a concentration two to five times greater than the plain nanoparticles in all types of tumors, regardless of whether they expressed the HER2 protein. They also found that the amount of tumoral trastuzumab-coated nanoparticles was even greater (10-fold) in mice that had a fully functional immune system and were bearing mouse-derived tumors. This led the researchers to suspect that the host animals’ immune systems were interacting strongly with the nanoparticles and playing a role in determining retention of the particles in the tumor, whether or not a drug was added. More experiments revealed that tumor-associated immune cells were responsible for collecting the
nanoparticles and that mice bred with an intact immune system re-
tained more of the trastuzumab-coated nanoparticles than mice
bred without a fully functioning immune system. Finally, the re-
searchers found that exposure to nanoparticles inhibited tumor
growth three to five times more than controls and increased CD8-
positive cancer-killing T cells in the tumors. Surprisingly, the anti-
cancer immune-activating response was equally effective with
exposure to either plain or trastuzumab-coated nanoparticles. Image
credit: Robert Ivkov, PhD. (Sci Adv. 6, eaay1601; https://doi.org/10.
1126/sciadv.aay1601.)