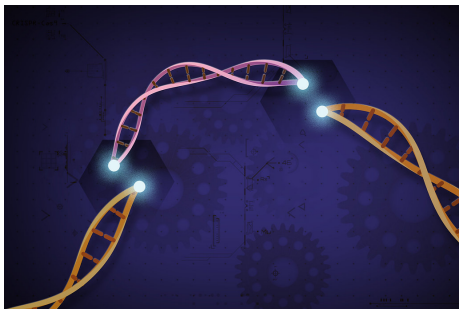

Inactivating viral replication with CRISPR/Cas systems

Value Proposition

Current gene therapy approaches based upon targeted DNA endoculeases, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), are based upon custom built DNA binding domains. Although gene therapy with ZFNs has progressed through clinical trials in several cases, they are only capable of targeting a single locus and are known to have low specificity. These technologies are unwieldy, difficult to execute, and are only capable of single target site cleavage. To target multiple DNA targets simultaneously, bacterial type II CRISPR/Cas9-based RNA-guided DNA endonucleases (RGNs) can be employed. These RGNs consist minimally of a Cas9 endonuclease loaded with a single guide RNA that is fully complementary to the desired DNA target sequence. In contrast to other targeted nucleases using custom engineered DNA binding domains designed to target a unique sequence, Cas9 proteins can be retargeted simply by expression of a distinct guide RNA. However, the Streptococcus pyogenes Cas9 RGN (SPCas9) greatly exceeds the packaging limit of ˜4.8 kb for adeno-associated virus (AAV)-based vectors, which are currently the preferred gene delivery vectors for in vivo purposes. There is a need for smaller Cas9 proteins to be developed.

Technology

Duke inventors have reported materials that incorporate novel Cas9 variants isolated from distinct bacterial species which exhibit similar specificity and efficiency to *S. pyogenes* Cas9, but are ~25% smaller at only ~3.2 kb in size. This invention is intended to treat viral infections or viral induced cancer. This enables the expression of these Cas9 variants from AAV vectors - which have a limited packaging capacity – while retaining over 1 kb in packaging space to incorporate sgRNA expression constructs. Additionally, this invention comprises a novel method for expressing sgRNAs using an alternative promoter and fusion-transcript system, reducing the space requirement from ~450 bp down to ~220 bp – a reduction of over 50%. The reported compositions solve the problem of low specificity by providing multiplex targeting to greatly extend editing efficiency beyond what is possible using ZFNs to permit not only gene disruption but also full gene deletion. In the context of virus-infected target cells, gene deletion could also be used for removal of a viral receptor or essential co-factor, rendering those cells refractory to infection. There are numerous tissues that are difficult to transduce where AAV is the sole capable option. It is in these tissues where CRISPR/Cas9/AAV will open up new gene therapy potentialities as an antiviral treatment option. This space-saving system has been demonstrated to perform similarly to standard CRISPR/Cas9 in *in vitro* assays in human cell lines and, as an antiviral therapeutic, reduces HBV viral loads and HPV-associated cancer progression in corresponding mouse models.

Other Applications

This invention potentially represents a “platform” technology on which a variety of genome engineering/gene therapy approaches may be based.

Advantages

- Provides a tremendous improvement in CRISPR/Cas9 by enabling expression from a single, safe and effective AAV vector
- Ability to express Cas9 and up to 5 sgRNAs from a single AAV, enabling simultaneous targeting of multiple sequences
- Overcomes the package size limitations of the AAV vectors by using the smaller Neisseria promoters
- Proof of concept studies have been successfully demonstrated with hepatitis B virus
- Has been patented for the removal of latent viral genomes (i.e. hepatitis B virus, herpes simplex virus, human papillomavirus) from infected cells, and could represent a potential cure for these otherwise lifelong diseases

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Publication(s)

External Link(s)

- From the lab of Dr. Bryan Cullen