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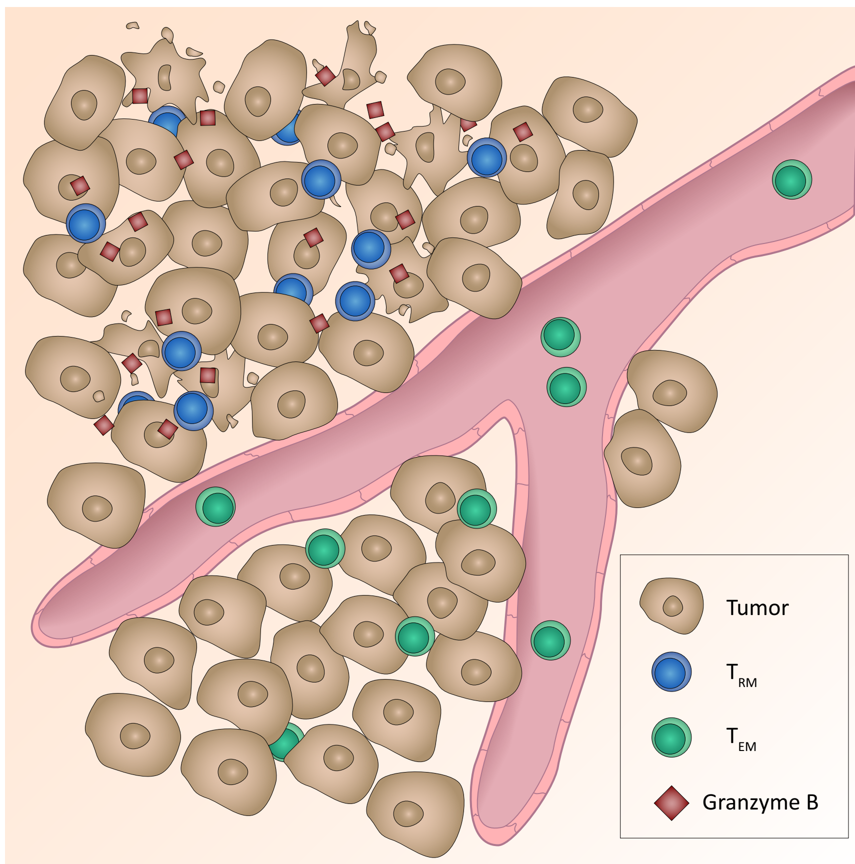

Figure 1. Effector Functions of Tissue-Resident Memory T Cells in the Breast. In triple-negative breast cancers, the tissue resident memory T cells have higher effector functions for killing tumor cells than T cells that enter the tissues.

tissue (as the authors demonstrated in a few cases of prophylactic mastectomies from healthy women). Nevertheless, in the age of immunotherapy, even rare anti-tumor immune populations may prove to be clinically important in deciding which patients to treat. Specifically targeting genes and pathways in T_{RM} cells in TNBC patients may improve responses in immunotherapy that, despite intensive investigation, have been underwhelming in clinical trials.

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References

1. Savas, P. *et al.* (2018) Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nat. Med.* 24, 986–993
2. Papalexi, E. and Satija, R. (2018) Single-cell RNA sequencing to explore immune cell heterogeneity. *Nat. Rev. Immunol.* 18, 35–45
3. Neu, K.E., Tang, Q., Wilson, P.C. and Khan, A.A. (2017) Single-cell genomics: approaches and utility in immunology. *Trends Immunol.* 38, 140–149
4. Tirosh, I. *et al.* (2016) Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* 352, 189–196
5. Mackay, L.K. *et al.* (2012) Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. *Proc. Nat. Acad. Sci. U. S. A.* 109, 7037–7042
6. Wang, Z.-Q. *et al.* (2016) CD103 and intratumoral immune response in breast cancer. *Clin. Cancer Res.* 22, 6290–6297
7. Jiang, X. *et al.* (2012) Skin infection generates non-migratory memory CD8 TRM cells providing global skin immunity. *Nature* 483, 227–231
8. Ali, H.R. *et al.* (2014) Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. *Ann. Oncol.* 25, 1536–1543

Spotlight

Emerging Strategies for Genome Editing in the Brain

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Despite the unparalleled therapeutic promise of genome editing, its curative power is currently limited by the substantial difficulty in delivering DNA-cutting enzymes to the cells in need of correction. A recent study demonstrates the potential for the delivery of pre-assembled genome-editing enzymes in the form of ribonucleoprotein complexes, which were used to rescue a mouse model of fragile X syndrome (FXS).

Genome editing has rapidly transformed biomedical research and has demonstrated therapeutic promise via successes in tissue culture, *ex vivo*, embryonic editing, and animal models of human disease. For successful

translation, a genome-editing therapeutic must be safe, effective, and ideally straightforward to manufacture. DNA encoding the RNA and protein components of a CRISPR-derived genome editing enzyme such as Cas9 can be delivered by adeno-associated virus (AAV) with high efficacy, but safety may be a concern and the manufacturing burden is substantial [1]. One emerging alternative is the delivery of genome-editing enzymes in the form of a pre-assembled ribonucleoprotein (RNA and protein, or RNP) complex. This approach is appealing because it ensures a tight therapeutic window: the RNP will be degraded in less than 24 h. By contrast, viral expression can result in prolonged expression of the genome-editing enzyme that persists for days. This has been associated with an increased prevalence of unintended off-target edits compared with RNP-based editing [2]. The nuclear localization signal (NLS) has routinely been used to ensure transport of RNP from the cytosol to the nucleus, but transporting a large genome-editing enzyme from the cell exterior to the cytosol presents a distinct challenge. Several strategies have been successful in promoting the cellular import of Cas9 RNP, such as modification of the Cas9 protein to include incidentally membrane-disrupting NLS sequences [3], or appending negatively charged domains to the Cas9 protein to promote its interaction with polymers that promote cellular entry [4]. The Murthy lab has developed an approach that uses a nucleating gold nanoparticle conjugated to single-stranded DNA to recruit Cas9 RNP, all of which is coated in a cationic polymer that facilitates delivery across the cell membrane, dubbed CRISPR-Gold [5].

The brain is an appealing site for initial forays into therapeutic genome editing because it is anatomically insular, allowing straightforward surgical access and providing an immunoprivileged status that ameliorates the risks associated with introduction of viral vectors [1] and/or genome-editing enzymes [6]. In a recent study by Lee and colleagues [7], the Lee and Murthy labs collaborated in using CRISPR-Gold to deliver either Cas9 or the analogous Cas12a (Cpf1) to the mouse brain. CRISPR-Gold carrying either Cas9 or Cas12a was stereotactically injected into the mouse hippocampus or striatum, performing efficient genome editing as detected by fluorescent reporters. A mouse model of FXS, based on an *Fmr1* knockout (KO), was used for experiments probing the ability of genome editing to treat autism. FXS is a common, inherited single-gene form of autism spectrum disorder (ASD), and drug treatments are largely inadequate. Importantly, the *mGluR5* gene has emerged as a promising candidate for genetic therapy, since it can contribute to FXS as well as other ASDs. To test whether reduction of mGluR5 could diminish autism-associated phenotypes in the FXS model mice, CRISPR-Gold bearing a Cas9 RNP targeting *mGluR5* was injected into the striatum. In treated striatal cells, 15% of *mGluR5* loci were disrupted, leading to a ~40% reduction in *mGluR5* mRNA or protein abundance, via qPCR or immunostaining, respectively. Behavioral studies of edited FXS model mice showed a marked reduction in two established hallmarks of mice with autistic phenotypes: marble-burying and spontaneous jumping. This promising result was bolstered by the observation that CRISPR-Gold treatment had no discernible impact on mouse locomotion. Other tests for toxicity showed that CRISPR-Gold treatment was not associated with cell death *in vivo* or changes in the properties of cultured neurons.

It is illustrative to evaluate these CRISPR-Gold results in comparison with AAV-mediated delivery, which was quickly adopted by pioneering genome editors for use in the brain (Figure 1). Delivery of AAV encoding Cas9 and its single guide (sg)RNA (Cas9/AAV) has been particularly successful in generating models of neurodegenerative diseases and other diseases of the brain and nervous system. A 2016 report from the Zhang laboratory reported Cas9/AAV-mediated editing of the *MCP2* gene in the brains of mice, resulting in disruptive edits in a majority (68%) of the cells in the injected tissue. The observed robust viral distribution throughout the tissue and editing in postmitotic neurons allowed generation of a mouse model of Rett's syndrome bearing the corresponding behavioral phenotype [8]. AAV has also been applied in therapeutic models; for example, the Davidson laboratory edited the disease-causing allele in a transgenic mouse model of Huntington's disease, observing reductions in the levels of mutant huntingtin protein of up to 80% following an injection of Cas9/AAV into the brain [9]. A similar approach was recently reported for *in vivo* editing of the mutant alleles of the *APP* gene that underlies Alzheimer's disease, another condition with dominant inheritance [10]. Cas9/AAV vectors were injected into the hippocampus of transgenic adult mice expressing multiple copies of the human mutant APP allele, and selectively generated indels (1.3%) in the mutant allele allowing a decrease in pathogenic amyloid-β protein levels in the brains of the mice [10]. Neither example of Cas9/AAV editing disease-causing mutant alleles demonstrated an associated therapeutic phenotype in mice, as was convincingly demonstrated with the CRISPR-Gold phenotype in the recent report by Lee and colleagues. However, the model systems differ, and it is reasonable to anticipate that Cas9/AAV-mediated editing might perform comparable editing in an FXS model system.

One apparent advantage of AAV-mediated delivery is that the viral particles

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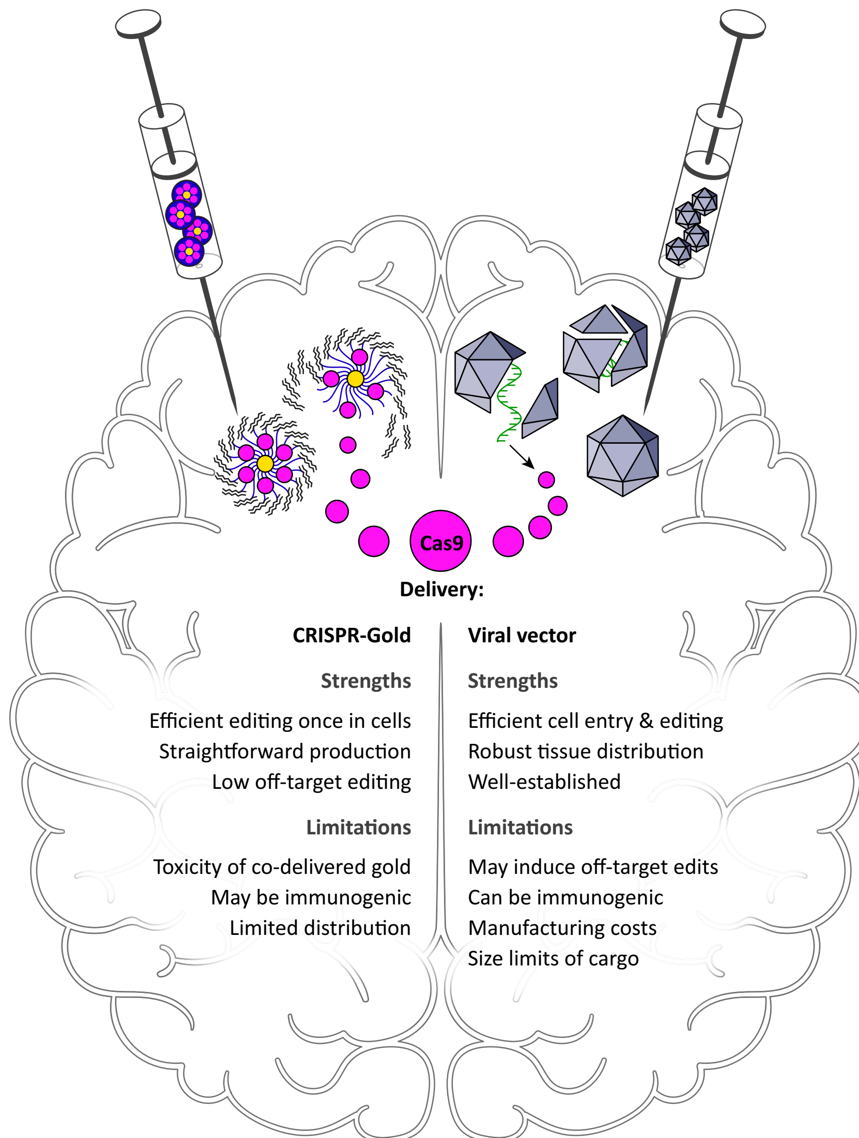

Figure 1. Delivery of Genome Editing Enzyme Cas9 to the Brain by CRISPR-Gold or Adeno-Associated Virus (AAV). Cas9 can be delivered to the brain in the form of an intact ribonucleoprotein complex (magenta) via CRISPR-Gold (left), or as DNA instructions (green) encapsulated within a vector, such as AAV. CRISPR-Gold comprises a gold nanoparticle (yellow) decorated with single-stranded DNA (blue) that recruits Cas9, all coated in a cationic polymer (black) capable of mediating cellular entry. By contrast, viral delivery relies on the capsid proteins (gray) for access to the cell interior, which is followed by transcription and translation of the packaged DNA to produce the RNA and protein components constituting Cas9.

spread throughout the brain in mice and primates. By contrast, RNP as delivered in isolation [3] or by CRISPR-Gold [7] tends to edit only cells within an area of several cubic millimeters. This suggests a potential hurdle for translation. Another potential concern related to the use of CRISPR-Gold in humans is its introduction of heavy metal, which is known to be toxic. However, this issue is tempered by the knowledge that the gold constitutes a miniscule fraction of the nanoparticle assembly by weight, and that genome editing is ideally a one-time treatment that avoids the accumulation of gold that would be associated with a treatment that is repeatedly dosed. With additional development, RNP delivery may prove itself as a leading strategy for therapeutic genome editing of the brain.

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https://doi.org/10.1016/j.molmed.2018.07.008

References

1. Colella, P. *et al.* (2018) Emerging issues in AAV-mediated *in vivo* gene therapy. *Mol. Ther. Methods Clin. Dev.* 8, 87–104
2. Kim, S. *et al.* (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Res.* 24, 1012–1019
3. Staahl, B.T. *et al.* (2017) Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat. Biotechnol.* 35, 431–434
4. Wang, M. *et al.* (2016) Efficient delivery of genome-editing proteins using bioreducible lipid nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 113, 2868–2873
5. Lee, K. *et al.* (2017) Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA *in vivo* induces homology-directed DNA repair. *Nat. Biomed. Eng.* 1, 889–901
6. Leong, Chew Wei (2017) Immunity to CRISPR Cas9 and Cas12a therapeutics. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 10, e1408
7. Lee, B. *et al.* (2018) Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours. *Nat. Biomed. Eng.* 2, 497–507
8. Swiech, L. *et al.* (2015) *In vivo* interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat. Biotechnol.* 33, 102–106
9. Monteys, A.M. *et al.* (2017) CRISPR/Cas9 editing of the mutant huntingtin allele *in vitro* and *in vivo*. *Mol. Ther. J. Am. Soc. Gene Ther.* 25, 12–23
10. György, B. *et al.* (2018) CRISPR/Cas9 mediated disruption of the Swedish APP allele as a therapeutic approach for early-onset Alzheimer's disease. *Mol. Ther. Nucleic Acids* 11, 429–440