Targeted Epigenetic Modification
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• TALE
  – Derived from plant pathogenic bacteria from the genus Xanthomas
  – One of many DNA-targeting proteins
  – Each repeat comprises 33-35 amino acids.
  – Can be rapidly synthesized to target any base pair sequence
  – Highly efficient and specific with minimal off-target effects

• CRISPR/Cas9
  – Original Cas9 derived from Streptococcus pyogenes
  – Uses a synthetic guide RNA (gRNA) to deliver the Cas9 to a desired location
  – Cas9 can be used interchangeably with different gRNA allowing for rapid targeting and flexibility

• Both systems can be constructed with a variety of transcription factors to epigenetically regulate gene expression (i.e., nucleases, activators, repressors)
Human HD fibroblasts confirmed to have SNP rs3857369 display significant allele-specific reduction of the mutant allele (red bar) without significantly altering healthy allele expression (blue bar).
Primary cortical and striatal neurons cultured from a 10 month old YAC128 mouse treated with TALE T3y.

Fink, KD
In vivo injection of the TALE plasmid and synthetic RNA using TurboFect and Invivofectamine.
Transcription Activator-like Effector (cont.)

Quantification of TALE biodistribution and expression following LNP encapsulation and unilateral injection.

Fink, KD
Co-localization of the TALE T3y with cortical and striatal neurons with TaqMan SNP genotyping for allele expression and Western Blot for protein quantification.
Transcription Activator-like Effector (cont.)

Toxicity screen following injection of LNP encapsulated TALE T3y.
Other Disease Indications

CDKL5 deficiency
TALE and dCas9

Angelman Syndrome
MSC Delivery of S100

Gene Activation:
Loss-of-function
- Angelman’s Syndrome
  - Zinc Finger approach
- CDKL5 deficiency (infantile epilepsy)
  - TALE and CRISPR activation studies

Gene Silencing:
Gain-of-function diseases or disorders
- Huntington’s disease
  - TALE and CRISPR silencing studies
- Potential genes implicated in cancer
Thank you!

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