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2017 CASRIP Innovation Policy Summer Institute

Latest Developments in CRISPR Technology

July 10, 2017

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Gene Therapy/Editing

- Early gene therapy approaches utilized wholesale replacement of defective genes
- Newer approaches utilize nucleases to introduce targeted double-strand breaks (DSBs) into genome, allowing for targeted insertion/deletion
- Nuclease strategies include:
  - Zinc Finger Nucleases (ZFNs): nuclease linked to zinc finger peptide that provides target sequence recognition
  - Transcription Activator-Like Effector Nucleases (TALENs): nuclease linked to TALE protein that provides target sequence recognition
  - Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas: Cas nuclease directed to target sequence by guide RNA (gRNA)

CRISPR/Cas System

- CRISPR/Cas identified as part of bacterial adaptive immunity system
  - DNA from invading virus cut into pieces and incorporated into CRISPR locus amid short repeats
  - Loci containing viral DNA transcribed and processed to generate CRISPR RNA (crRNA)
  - crRNA complexes w/ Cas nuclease and trans-activating crRNA (tracrRNA) to cleave foreign DNA containing viral sequence
  - Cas9 contains two nuclease domains, RuvC-like and HNH-like, allowing for cutting of both DNA strands to generate double-strand breaks (DSBs)
- CRISPR/Cas gene editing utilizes synthetic guide RNAs (gRNAs) containing a targeting sequence and tracrRNA components
- Use of RNA for targeting makes CRISPR/Cas system cheaper, more efficient than other nuclease approaches

Potential Uses of CRISPR/Cas System

- Therapeutic gene editing
  - Introduction of indels or deletions to disrupt target genes associated w/ disease
    - DSB repaired by non-homologous end joining (NHEJ)
  - Correction or replacement of target sequences in faulty genes
    - DSB repaired by homology-directed repair (HDR) using "correct" template sequence
  - Editing can take place *ex vivo* or *in vivo*
  - Can target gene or its regulatory elements
- Screening for gene/domain activity
- Generating engineered microbes, crops, etc.
- Animal modifications, e.g., anti-malarial mosquitoes
- Sensitizing bacteria to antibiotics

Clinical Trials

- China at the forefront, numerous trials already underway or in late planning stages
  - First human CRISPR trial started Oct. 2016 (Sichuan Univ.)
    - *Ex vivo* disruption of PD-1 gene in immune cells from lung cancer patients
  - Numerous additional trials targeting PD-1 in different cancer types in early stages
  - HPV trial slated to start this month (Sun Yat-Sen Univ.)
    - *In vivo* disruption of viral genes using gel applied directly to cervix
  - Several CAR-T trials in late planning stages
    - Editing T cell genes to make cells target specific cancers
- First US clinical trial approved by NIH mid-2016, awaiting FDA greenlight (Univ. of Penn)
  - *Ex vivo* disruption of PD-1 gene and T cell primary receptors, coupled with addition of NY-ESO-1 receptor using traditional gene therapy

Complicated Patent Landscape

- Broad Institute of MIT and Harvard (Zhang) and Univ. of CA, Berkeley (Doudna/Charpentier) currently battling over foundational CRISPR patents
  - US: Broad prevailed in patent interference Feb. 2017 on basis that its eukaryotic methods were patentably distinct from UC's methods
    - UC currently appealing to the Federal Circuit
  - EP: EPO announced intent to grant broad patent to UC Mar. 2017
    - Broad and others expected to oppose
  - CN: SIPO announced intent to grant broad patent to UC this week
  - Uncertainty of foundational patent rights complicates licensing
- Broad, UC, and numerous other entities aggressively pursuing "second generation" patent applications

Next Steps

- Despite its promise, there are many issues to be worked out to enable widespread therapeutic use of CRISPR editing
  - Reduce off-target effects, e.g., by improving target sequence selection, gRNA optimization
    - Recently reported that mice cured of blindness using CRISPR exhibited numerous off-target mutations
  - Develop improved methods of delivery, e.g., vectors, viral and non-viral delivery systems
    - Particularly challenging for *in vivo* methods
  - Improve nuclease performance and specificity, e.g., using modified Cas or novel nucleases
  - Develop “shut off” switches to prevent unwanted editing
- There are also clear ethical issues to consider with regard to certain types of gene editing